ON THE MECHANISM OF THE EFFECT OF MALEIC HYDRAZIDE ON PLANTS

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Maleic hydrazide (MH) is one of the most potent of the regulators of plant growth and development which have wide practical application [1].

One of the most important uses for MH seems to be the prevention of growth of tuber and root crops during the period of storage. Besides this, MH is used successfully to prevent the growth of lateral buds in tobacco, as a herbicide, and also to bring about male sterility in plants in the process of producing hybrid corn. A big possibility for the use of MH in plant culture results from its ability to bring about a temporary cessation of plant growth. Though, in most cases, plants treated in this way do not in the final analysis show decreased yield, but only some narrow and deformed leaves, yet in fruit trees the yield of fruit is significantly decreased [2]. Removal of the harmful aftereffects of MH would markedly widen the practical applicability of this substance, but this possibility will only be realized when the mode of action of MH is understood.

In view of this, investigations on the mode of action of MH, since they look forward to a time when this chemical may have great practical use, take on not only great theoretical but also practical importance.

Up to the present time, several different theories have been proposed as to the mode of action of MH. That MH works as an anti-auxin, as was first proposed in 1951 [3], is the hypothesis which has been most widely accepted. More recently, the anti-auxin action of MH has been explained as its influence on the metabolism of indole-acetic acid in plants [4-6], and in some papers [7,8] as a stimulation of the enzyme systems which oxidize IAA, especially IAA oxidases or peroxisases. Experiments carried out in vitro on the basis of this hypothesis by Kenten [7] and Andreae [9] show an increase in the activity of peroxidases in the presence of MH. It seems from these experiments that MH is a substrate of peroxidase. These demonstrations of the stimulatory effect of MH on oxidative enzymes which destroy IAA were mostly obtained in vitro with plant extracts, or with purified preparations of peroxidase. However, investigations of the effect of MH on auxin metabolism in whole plants yield results which do not support the anti-auxin hypothesis. Particularly noteworthy are the results obtained by Audus, working with peas, beans, and sunflowers [10]. In his experiments, the addition of a typical anti-auxin (2, 3, 5-iodobenzoic acid) reduced the amount of heteroauxin in the plants 10,000 times, while in the same period the addition of MH caused even some increase in the level of heteroauxin.

In experiments with bean seedlings, Pilet [11] found that high concentrations of MH caused an increase in the activity of IAA oxidase, but much lower concentrations, while bringing about a strong inhibition of growth, did not affect the activity of IAA oxidase.

Butenko (unpublished results, 1956) showed that treatment of 10-day-old corn, soybean, and wheat seedlings with a 0.5% solution of the diethanolamine salt of MH did not decrease (corn) or even somewhat increased (soybean and wheat) the amount of free IAA in the tissues of these plants.

The effect of MH on the oxidation-reduction system in plants has been studied by many authors. Naylor and Davis [12] and Isenberg [13] have observed an inhibition of plant respiration resulting from treatments with high concentrations of MH. Currier and Zweep [14] reported an inhibitory effect of MH on the reduction of tetrazolium by dehydrogenase. All these facts, and also deductions from the structure itself of MH, enabled Muir and Hansch [15] to propose the first chemical hypothesis explaining the mode of action of MH. Regarding MH as a derivative of maleic acid containing a double bond and activated by two carbonyl groups, Muir and Hansch suggested that it might combine with the greatly active sulfhydryl groups of some enzymes, especially succinic dehydrogenase, by coupling with them at the double bond. This type of coupling reaction has been observed earlier with maleic acid and mercapto-acetic acid. Weller, Ball, and Sell [16] tried to provided experimental proof of this hypothesis. However, these authors obtained negative results, and found that MH did not react with mercaptoacetic acid, cysteine, or glutathione, even in the presence of homogenates from radish leaves. They also took issue

with the recent theories about the molecular structure of MH, which, according to spectrographic studies, exists in the oxypyridazone form, where it does not have an activated double bond.

Between 1951 and 1955, some work by Greulach was published which was devoted to the search for anti-metabolites of MH. In the first paper [17], starting from the hypothesis of the anti-auxin effect of MH, the author took as anti-metabolites various synthetic growth-promoting substances—indolebutyric and α -napthylacetic acids--as well as several other growth-regulating substances, but none of these substances reversed the inhibitory effect of MH on plant growth. According to Greulach, however, MH interacts with succinic, fumaric, and nicotinic acids, with various aminoacids, and also with thiamine, fructose-triphosphate, coconut milk, $FeCl_3$, $CoCl_2$, pyrimidines and purines—thymine, uracil thiouracil, hypoxanthine, adenine, adenosine, ribose, DNA and RNA [18]. A partial relief of the inhibitory effects of MH on germinating peas was obtained by the author by using a complete mineral medium, and also in the presence of thymine, uracil, and thiouracil. However, these results did not permit Greulach to draw any conclusions as to the mode of action of MH.

One of the authors of the present work (see [19]) formulated the hypothesis that MH is an antagonist of a natural plant metabolite – uracil. The author drew attention to the close similarity in structure of MH and uracil, the molecules of which differ only in the position of the carbonyl groups and the atoms of nitrogen in the ring, and also to studies of the effects on plants of another analogue and antagonist of uracil [20, 21]—thiouracil—and to the almost complete lack of physiological activity in the simplest derivatives of MH [22]. According to his hypothesis, MH replaces uracil in the biosynthesis of nucleic acids in plants, and at the same time blocks their activity. The replacement of carbon atoms in the MH molecule apparently makes it difficult for simple MH derivatives to enter any active complex, which explains their weak physiological activity as inhibitors of plant growth. There are many results reported in the literature which are in accord with this hypothesis. Thus, in investigations of the effects of MH on growth processes, it has been shown to cause frequent abnormalities in the process of mitosis in plant meristems [23]. These abnormalities bring about the inhibition, and high concentrations of MH lead to complete disruption of the cell division process, which is directly connected with the synthesis of nucleic acids in the cell.

Another finding, which suggests that the primary action of MH is on the synthesis of nucleic acids, would seem to be the unpublished experimental results obtained in 1956 by Butenko with corn seedlings, fully showing the effect of MH on the incorporation of labelled phosphorus into the nucleoprotein fraction. In these experiments, the activity of P^{32} in the nucleoprotein fraction of the control plants was 400 counts/min/100 mg dry weight, while no incorporation of labelled phosphorus was observed in the same period of time in plants treated with MH, though in the acid-soluble fraction and mineral phosphorus, the activity in the MH treated plants was even somewhat higher than in the controls (63,000 as against 46,000 counts/min). Recent experiments on tomato plants have shown the existence of an antagonism between uracil and MH in plants [24]. However, under the experimental conditions used, the authors were not able to obtain a complete reversal of the effects of MH with uracil.

For the further investigation of the mode of action of MH, we used the method of plant tissue culture. One of the advantages of this method seems to be the relatively simple response of the tissue to the treatment, compared to the responses of the whole plant, where the primary changes in metabolism evoke multiple correlative changes in other processes of the over-all metabolic system of the whole organism. Besides this, the occurence of these secondary processes can change according to the particular external conditions in which the plant is growing, and according to the physiological state of the plant itself. The culture of isolated tissues of plants, since it makes it possible to control the nutrient medium and the enviromental conditions in which the plant is growing, seems to be a good method to use in studying the specific mode of action of physiologically active substances on the metabolic processes and growth of plant tissues.

Tissue culture has already been used in the study of the mode of action of MH. Kulechs [25], who worked in Gautheret's laboratory, studying the interaction of MH and auxins, showed in cultures of Jerusalem artichoke tissue that even high concentrations of MH, equal to 0.1 g/liter, which strongly inhibited the growth of the tissues, did not affect the synthesis or concentration of auxins in them. Morel [26], examining the effect of MH on the activity of the oxidative enzymes, found that MH decreased the activity of peroxidase.

In our experiments, tissue culture was used for a complete study of the interaction of MH with uracil, sodium ethylxanthogenate, and riboflavin. The experiments with sodium ethylxanthogenate were carried out to test the hypothesis of Muir and Hansch that this compound contains a sulfhydryl group, and according to them can combine with the MH molecule at the double bond and thus inactivate it. Information [27] about the photo-oxidation of MH induced by riboflavin, and also published experimental results showing that it partially relieved the inhibitory effects of MH on the growth of tomato plants [24], led us to employ it in our experiments.

METHODS

Experiments to study the effect of MH and of its possible antagonists in growth were carried out with callus culture of carrot. This tissue was isolated in 1938 in France in Gautheret's laboratory, and was obtained by

TABLE 1. The Effect of MH and Uracil Incorporated in the Nutrient Medium on the Growth of Carrot Callus Tissue

No. of expt.	Addition to basal medium, g/liter	Fresh wt. callus, mg	% dry wt.
1	Without addition (control)	1060	6.6
2	Uracil (1×10^{-2})	1320	6.3
3	MH (1×10^{-4})	1420	8.4
4	MH (1×10^{-4}) + Uracil (1×10^{-2})	1240	7.2
5	MH (1×10^{-3})	1090	7.6
6	MH (1×10^{-3}) + Uracil (1×10^{-2})	1060	7.2
7	MH (5×10^{-3})	570	8.6
8	MH (5×10^{-3}) + Uracil (1×10^{-2})	1080	7.6
9	MH (1×10^{-2})	180	9.2
10	MH (1×10^{-2}) + Uracil (1×10^{-2})	200	9.1
11	MH (1×10^{-1})	100	9.4
12	MH (1×10^{-1}) + Uracil (1×10^{-2})	150	9.3

us through the kindness of E. Chesnovskov (Poland), to whom we express our deep gratitude. The callus tissue of carrot (Fig. 1) exists as a physiologically related clone, characterized by rapid undifferentiated growth, becoming green in the light, and not requiring auxin in the nutrient medium for its growth. Therefore this tissue belongs to the so-called "habituated" type. The callus was grown on Nitsch's agar medium [28] with the following composition:

Compound	Concentration, mg/liter
KNO_3	2 000
KCl	1 500
$MgSO_4 \cdot 7H_2O$	250
$NaH_2PO_4 \cdot H_2O$	250
$CaCl_2$	25
Sucrose	20 000
Agar	8 000

To this solution was added Heller's micronutrient supplement [29]. According to our results, carrot callus tissue grows better on this than on Heller's medium. The callus was grown in bottles 23 x 150 mm. There were 10-12 treatment replications used. Each experiment was carried out twice. The callus was grown in the dark in a controlled growth chamber of the Artifical Climate Station of the K. A. Timiryazev Institute of Plant Physiology, at a constant temperature of 26°C and a relative humidity of 70%. In other experiments, where the influence of light on the activity of chemical substances dissolved in the solution was examined, the callus was grown in special boxes with daylight fluorescent bulbs. The intensity of illumination in these boxes was 13 000 ergs/ /cm^2/sec. The temperature under the bulbs was 24°C. The dark control employed here was put underneath the bulbs in a black lightproof container.

Portions of callus weighing 20-40 mg were used for the most part in the experiments, the callus in the controls increasing to about 1000 mg in the course of 1-1.5 months. The chemical substances to be tested were dissolved in the nutrient medium before it was autoclaved, since according to experimental results already obtained they were thermostable. Experiments were begun with the diethanolamine salt of MH, but since this salt is inhibitory to plant growth, the free acid form of MH was used in all subsequent experiments. At the termination of the experiment, the pieces of callus were weighed, photographed, and for each treatment the dry weight was determined.

EXPERIMENTAL RESULTS

The growth of carrot callus tissue in the presence of different concentrations of MH in the nutrient solution is shown in Fig. 2, I. At low concentrations (1×10^{-6}–1×10^{-4} g/ liter somewhat stimulates the growth of tissue, but beginning at a concentration of 2×10^{-3} g /liter and at

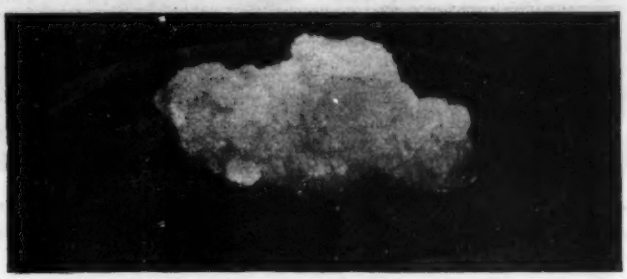

Fig. 1. Callus tissue of carrot, grown for 45 days in the dark.

higher concentrations, it inhibits and causes an almost complete cessation of growth. Determination of the dry weights of the tissues shows that all concentrations of MH tested decreased the hydration of the callus tissue (Table 1).

MH inhibits the growth of tissue not only when it is directly incorporated in the medium, but also when the pieces of callus are subsequently transferred onto a medium not containing MH. In one experiment, we observed a strong inhibition of growth, equal to the original one, through five transfers of tissue on medium not containing MH, i.e., during a period of 10 months after direct treatment with MH. This phenomenon (aftereffects of MH) can be explained either as the existence of enough residual MH through the course of transfer of tissue to bring about a stoppage of growth, or, more likely, as irreversible damage to those metabolic processes connected with the growth of the tissue, resulting from the initial effect of MH.

In examining the interaction of MH with uracil, we first studied the effect of uracil itself on the growth of the carrot tissue when dissolved in the nutrient solution at different concentrations. In Fig. 2, II are shown the effects of different concentrations of uracil. Its activity curve has a shape typical of physiologically active compounds. Low concentrations slightly stimulated, intermediate concentrations seemed to be optimal, and high concentrations inhibited growth. Using uracil as a antagonist of MH, the effects of one stimulatory level (1×10^{-2} g/liter) and one inhibitory level (5×10^{-2} g/liter) were examined. However, in the interaction of uracil and MH, no difference between these concentrations was observed, and in all further experiments a concentration of 1×10^{-2} g/liter uracil was used.

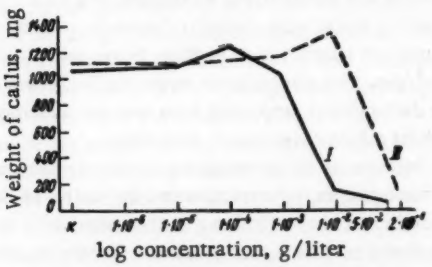

Fig. 2. The effect of MH (I) and uracil (II) on the growth of carrot callus tissue

From the numerical results in Table 1 and from Fig. 3, it may be seen that when both MH and uracil are added to the culture medium, no synergism occurs between them at concentrations of MH which are stimulatory to growth; on the other hand, while growth in the presence of both compounds is greater than in the controls, it is somewhat less in comparison with the growth of tissues in the presence of either MH or uracil alone. However, at concentrations of MH which are inhibitory to growth, the effect of uracil becomes marked. This effect is especially marked at 5×10^{-3} g/liter MH. This concentration of MH causes a 50% inhibition of growth in experimental plants as compared with the controls, but the addition of uracil to the medium in this case brings about a complete restoration of growth. Apparently, at this concentration the molecular ratio between MH and uracil is most favorable, and RNA synthesis, while still somewhat decreased by MH,

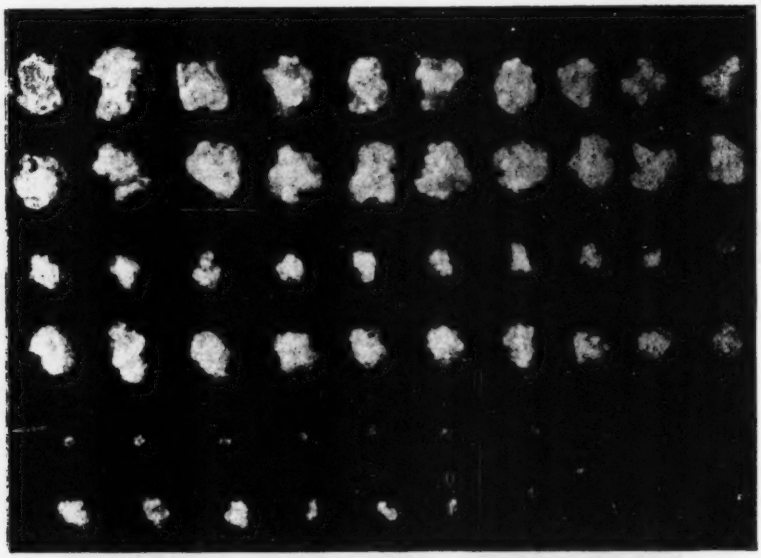

Fig. 3. The effect of MH and uracil on callus growth when added to the nutrient medium. From the top: 1) Basal medium alone; 2) 1×10^{-2} g/liter uracil; 3) 5×10^{-3} g/liter MH; 4) 5×10^{-3} g/liter MH + 1×10^{-2} g/liter uracil; 5) 1×10^{-2} g/liter MH; 6) 1×10^{-2} g/liter MH + 1×10^{-2} g/liter uracil.

TABLE 2. The Effectiveness of Uracil in Reversing Growth Inhibition Brought About by Pre-treatment with MH

No. of Expt.	Concentration of MH in medium before transfer of tissues, g/liter	Mean weight of callus in mg/ transfer	
		on basal medium	on basal medium + 1×10^{-2} g / liter uracil
1	Control (no treatment)	1020	1330
2	MH (1×10^{-4})	1200	1200
3	MH (1×10^{-2})	200	610
4	MH (1×10^{-1})	20 (No Growth)	160

TABLE 3. The Effect of MH and Riboflavin on the Growth of Carrot Callus Tissue, when Added Alone or in Combination to the Nutrient Medium

No. of Expt.	Addition to the basal medium, g/liter	Mean wt. of callus, mg
1	Control	1500
2	Riboflavin 1×10^{-3}	2700
3	MH 8×10^{-3}	560
4	MH 8×10^{-3} + Riboflavin 1×10^{-3}	360
5	MH 1×10^{-2}	460
6	MH 10^{2} + Riboflavin 1×10^{-3}	300

reaches a maximal rate. At higher MH concentrations the effect of uracil, though noticeable, is not as great.

The results of experiments in which the effect of uracil following treatment with MH was investigated are particularly interesting. In these experiments, tissues which had been growing on a medium containing MH were transferred onto a medium without MH. As may be seen from Table 2 and Fig. 4, tissues transferred from a medium containing 1×10^{-1} g /liter MH onto the control medium did not grow, while those transferred onto a medium containing uracil grew to five to six times their original weight. In another experiment, where the tissues were transferred from a medium containing 1×10^{-2} g/ liter MH onto a medium containing uracil, growth was three times that on the control medium.

The experiments with sodium ethylxanthogenate and riboflavin were carried out in the same way as those with uracil. Sodium ethylxanthogenate, while stimulating growth of callus tissue at a concentration of callus tissue at a concentration of 5×10^{-2} g /liter, was completely without effect when it was added to the medium with various concentrations of MH (Fig. 5). Riboflavin, in concentrations of from 1×10^{-5} - 5×10^{-2} g /liter, decreased the growth of callus tissue markedly, both in light and dark(Fig. 6). The effect of riboflavin was particularly noticeable on tissues growing in the light, but when it was added to the culture medium together with MH (Table 3), riboflavin not only did not stimulate

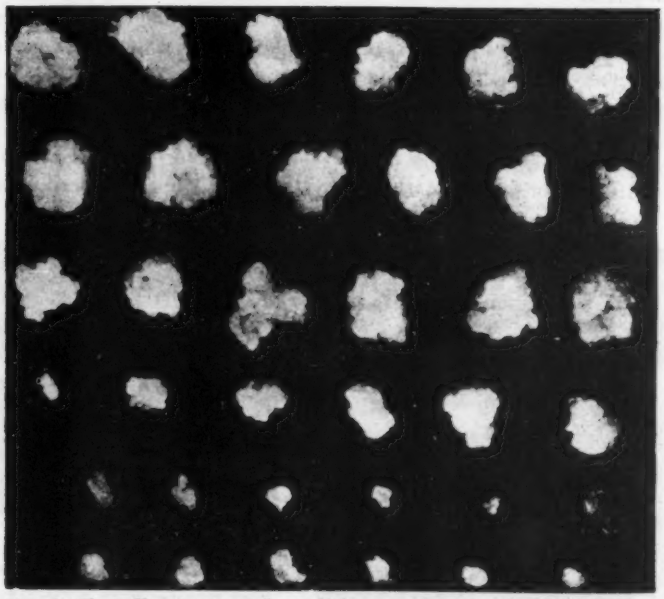

Fig. 4. The effectiveness of uracil in reversing growth inhibition brought about by pre-treatment with MH. From the top: 1) Basal medium alone; 2) 1×10^{-2} g /liter uracil; 3) 1×10^{-2} g /liter MH followed by transfer to 1×10^{-2} g /liter uracil; 4) 1×10^{-2} g /liter MH before transfer; 5) 1×10^{-1} g /liter MH before transfer; 6) 1×10^{-1} g /liter MH followed by transfer to 1×10^{-2} g /liter uracil

TABLE 4. The Effect of Riboflavin on the Aftereffects of MH

No. of Expt.	Conc. of MH in medium before transfer, mg/ liter	Mean weight of callus in mg/ transfer			
		on basal medium		on basal medium + 1×10^{-3} g/liter riboflavin	
		light	dark	light	dark
1	Control	80	890	1200	600
2	MH 8×10^{-3}	30	40	20	20
3	MH 1×10^{-2}	20	20	20	20

tissue growth in either light or dark, but even somewhat intensified the inhibition caused by MH. The same result was observed when tissues were transferred from a medium containing MH to one containing riboflavin, either in the light or the dark (Fig. 7 and Table 4). It is interesting to point out that uracil acts as an antagonist of MH either in light or dark, as was shown by special experiments (Fig. 7). Thus, the experiments with riboflavin have shown that it does not seem to be an antagonist of MH in the case of carrot tissue cultures. It is possible that these results apply only to the type of tissues used in our experiments, and differ from those obtained in experiments where whole plants have been used. This might be the reason for the disagreement of our results with those in the literature [24] concerning the interaction of MH and riboflavin in tomato plants.

The experiments with sodium ethylxanthogenate do not substantiate the hypothesis of Muir and Hansch about the mode of action of MH.

Therefore, the experimental results obtained support the proposed hypothesis [19] as to the antagonism between MH and uracil, and give indirect evidence for the blocking of nucleic acid synthesis in plant tissues by MH. However, further research is needed to show the primary effects of MH on nucleic acid metabolism.

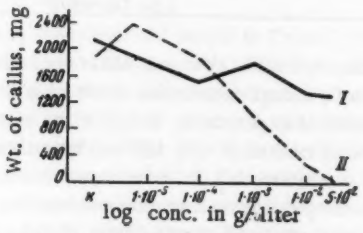

Fig. 6. The effect of riboflavin on growth of carrot tissue in dark (I) and light (II).

SUMMARY

Introduction of 10^{-2} g/liter of uracil into a nutrient medium containing maleic acid hydrazide (MH) at a concentration ($5 \cdot 10^{-3}$ g/liter) sufficient for inhibiting the growth of an isolated callus tissue of carrot restores the growth rate to its initial value. Tissues whose growth was appreciably inhibited in media containing large amounts of MH (10^{-2}–10^{-1} g/liter) began to grow at the same or an

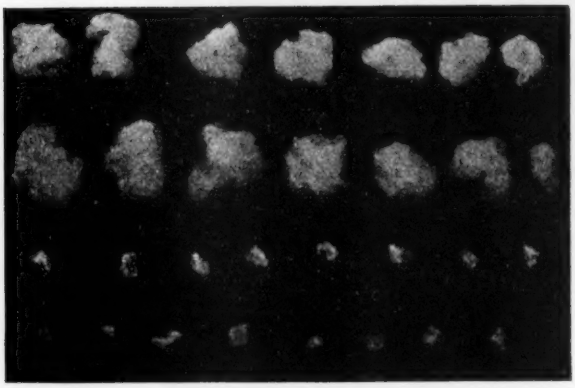

Fig. 5. The effect of sodium ethylxanthogenate on growth of carrot callus tissue. From the top: 1) Basal medium—control; 2) 5×10^{-5} g/liter sodium ethylxanthogenate; 3) 1×10^{-3} g/liter MH; 4) 1×10^{-3} g/liter MH + 5×10^{-5} gm/liter sodium ethylxanthogenate

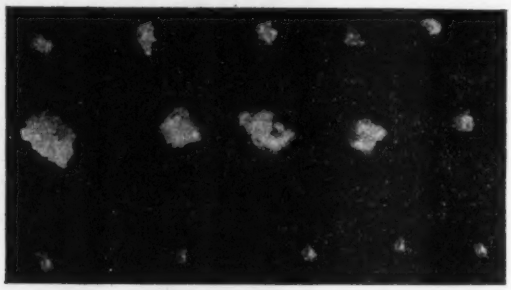

Fig. 7. The effect of riboflavin and uracil on growth, after transfer from a medium containing MH (tissues grown in the light). From the top: 1) 8×10^{-3} g /liter MH before transfer (grown on basal medium—control); 2) 8×10^{-3} g/liter MH, followed by transfer to 1×10^{-3} g/ liter uracil; 3) 8×10^{-3} g /liter MH, followed by transfer to 1×10^{-3} g /liter riboflavin in the culture medium.

even higher rate after transfer to a medium containing uracil.

Sodium ethylxanthogenate and riboflavin did not influence tissue growth which had been inhibited by MH. This was true whether this substance was introduced together with MH or after application.

The data obtained confirm the hypothesis regarding the antagonistic interaction between MH and uracil with respect to growth of plant tissues, and indirectly suggest a blocking action of MH on the biosynthesis of nucleic acids in plants.

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*See English translation.

FURTHER INVESTIGATION OF WATER EXCHANGE IN PLANTS USING H_2O^{18}

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The results of investigations of the influx of H_2O^{18} from nutrient solution into the roots, stems, and leaves of Phaseolus have been reported earlier [1]. At that time, the water metabolism of the organs of Phaseolus was studied by exposing the plants for periods varying from 15 minutes to 24 hours. The features of H_2O^{18} influx for exposure periods shorter than 15 minutes and longer than 24 hours were not tested. Since then, the investigation of H_2O^{18} influx during much shorter exposures, particularly the flux into the roots when just 15 minutes after submersion in nutrient solution the roots are nearly saturated with the heavy water, has become interesting, since this method could give a more precise insight into the dynamics of the exchange between root water and the nutrient solution. On the other hand, exposure for longer than 24 hours would test the persistence of the short-exposure distribution of H_2O^{18} between plant organs.

The objective of this paper is to fill in these gaps to a certain extent.

METHODS

Phaseolus vulgaris was used in the investigation. A few experiments were conducted with Mimosa pudica. The beans were raised in either quartz sand (Fig. 1) or soil (Table 1) in the greenhouse under artifical light (about 15,000 lux). The roots of plants 12-13 days old were washed free of sand and soil, the surface of the roots blotted with filter paper and the roots submerged in Knop nutrient solution containing H_2O^{18}.

After the desired exposure period, the plants were removed from the nutrient solution, the roots again carefully blotted dry with filter paper, and the plants dismembered into roots, stems, and leaves. These parts were analyzed for the isotope separately. In certain cases only the roots were taken in the experiment.

In order to determine the O^{18} concentration of the water contained in the various plant organs, the organs were freeze-dried under vacuum and the water captured. One milliliter of this water was introduced for isotopic exchange with a certain amount of gaseous carbon dioxide [2]. The isotopic composition of the oxygen of the carbon dioxide was determined by the mass spectrograph MI-1305 as the ratio of mass 46 to mass 44. The figures in the table indicate the experimental values for O^{18} contained in the analyzed samples compared to natural carbon dioxide, which we assumed to have 0.21 at.% O^{18}.

It is evident from Fig. 1A that, particularly during the initial minutes after submersion in the nutrient solution containing H_2O^{18}, a highly dynamic replacement of root-tissue water by an influx of outside water is observed. After one minute nearly 50% of all exchangeable water in the roots appears to have been replaced by water from the nutrient solution. After 7 minutes practically all of the exchangeable water of the root tissues has been replaced.

The rate of water exchange in the roots of detopped plants was about the same (Fig. 1B). This indicates that this high rate of water exchange in the roots is not a result of unidirectional water movement from the roots to the shoot but is chiefly a result of a trend toward root–solution equilibrium.

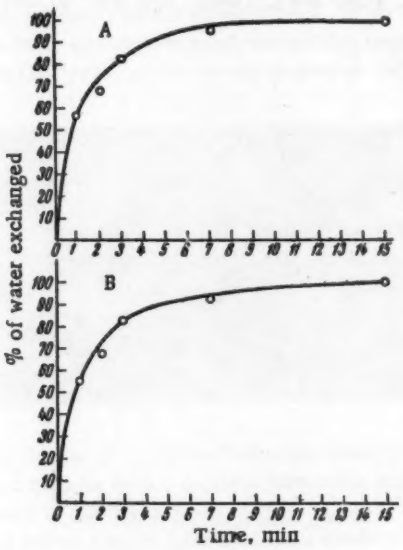

Fig. 1. Rate of water exchange in intact (A) and detopped (B) plants, assuming 100% of all root-tissue water has been exchanged (after 15 minutes).

TABLE. Extent of Water Replacement in Various Plant Organs During Protracted Influx of H_2O^{18} from an External Solution

Organ	Phaseolus vulgaris			Mimosa pudica
	21 hr	48 hr	75 hr	10 hr
Root	0.81	0.88	0.93	0.59
Stem	1.22	—	1.30	0.81
Leaf	0.58	0.82	1.05	0.59

Note: H_2O^{18} concentration in nutrient solution was 1.30 (Phaseolus vulgaris) and abour 0.9 (Mimosa pudica).

In subsequent experiments bean plants were held in nutrient solution containing H_2O^{18} for appreciably longer periods (see table). From the data in the table it is apparent that the over-all pattern of H_2O^{18} distribution in the organs of the bean which we previously found [1] for much shorter exposure times was also maintained when the plants were kept for longer times in nutrient solution containing H_2O^{18}.

Even after 75 hours in H_2O^{18}, the O^{18} content of leaves and roots was only 70-80% of the external solution. The O^{18} content of the stem equaled or nearly equaled the concentration in the external solution.

This pattern is apparently not characteristic of Phaseolus vulgaris alone. In a few experiments with Mimosa pudica the same sort of distribution of H_2O^{18} was observed (see table). Since the plants in this experiment were kept in the nutrient solution for only 10 hours it is apparently not possible to be certain that the leaves have come to equilibrium with respect to H_2O^{18}. However, the general patterns of H_2O^{18} distribution among the plant organs are retained here also.

Not long ago we obtained news of Gubner's research on the uptake of HDO by organs of Vicia faba*. He came to the conclusion that the lower concentration of heavy water in the roots and leaves occurred because of exchange between the water of these organs and the water vapor of the air. Therefore, in a plexiglas cabinet we set up still another series of experiments on protracted entry of H_2O^{18} into the organs of Phaseolus. In these experiments the opportunity for atmospheric water vapor to exchange with plant water was severely restricted because water vapor was continously removed by P_2O_5. Despite these conditions the O^{18} content of the leaves and roots was markedly lower than in the nutrient solution, while at the same time, just as in the preceding experiment [1], the concentration of heavy water in the stem nearly reached that of the external solution.

At present we are studying in more detail the possibility of rapid exchange between atmospheric water vapor and water in the plant organs.

SUMMARY

The water exchange rate and degree of substitution of water in plant organs during brief or prolonged uptake of H_2O^{18} from an external nutrient solution has been investigated.

A high rate of water exchange has been observed in bean plant roots detached from the shoot as well as in roots not detached from the shoot. After a 60-second submersion in the nutrient medium, about half of the exchangeable water of the roots was replaced by the external water.

After the plants were kept in H_2O^{18} over a very long time (up to 75 hours) the concentration of H_2O^{18} in roots and leaves was only 70-80% of that in the external solution, whereas in the stem tissues the H_2O^{18} content was almost the same as that of the external solution.

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† See English translation.

THE STATE OF WATER IN THE PLANT CELL

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A few studies recently published have dealt with the question of the variable influx of heavy water into plant tissues [1,2, etc.]. These workers have established that, when the plant is submerged in D_2O, T_2O or H_2O^{18} the tissues are enriched with the isotopic form of the water to the extent of 45-60% of the external concentration, depending on the organ involved.

Their studies have elicited two hypotheses from these investigators. One hypothesis concerns the possibility of an isotope effect, the other the presence of difficultly exchangeable water in the plant cells. Neither hypothesis unfortunately, answers the question as to where the observed separation occurs. By obtaining correlative data on the influx of D_2O into plant tissues we set about to clarify the reasons for such a nonuniform enrichment of plants with labeled water.

It has already been shown [3] that the mobility of water in the vicinity of a swelling colloid is quite great. Motion of the protoplasm itself undoubtedly indicates that such a partition of water cannot be explained by the presence in the plasma of so-called bound and free water particularly since, in view of the dynamic state of the protoplasm, we must also admit that the water within it is also in a mobile state.

After perusing data obtained on the enrichment of plant tissues with heavy water we hypothesized that the spatial partition of the water took place in the plant cells and that the fraction of it with low mobility is found in the vacuoles.

We conducted the following experiments in order to test this proposition.

Nitella plants were transferred from ordinary water to water enriched with D_2O solution for one-quarter, one and a half, and 25 hours. After exposure, the Nitella cells were speedily blotted with filter paper and analyzed with an interferometer. Accuracy of analysis was 0.2% for D_2O. The results are shown in Table 1.

Just as expected, tagged water entered the plant cells rapidly and the enrichment level remained constant over a long period. In this case the enrichment was somewhat more than 60% of the external concentration of heavy water used.

However, if we really are to verify the hypothesis that the water in the cellsap and plasma are partitioned relatively simply through the tonoplast and that water in the vacuole is only slightly mobile, then by destruction of this barrier we ought to obtain the same percentage of D_2O as in the protoplasm; this is close to that of the external water. No apparatus recommended for separation of cell sap from plant tissue was suitable in this case since the use of such crude methods as centrifugation, pressing out the sap, plasmolysis, etc., inevitably destroy the protoplasmic structure and alter the filtration of water through it from the vacuole.

The large cells of Nitella permitted us to carry out this separation, i.e., we could find the percentage of D_2O in the protoplasm by simply dissecting the cells.

The experiment was set up in the following way. Nitella cells were submerged in a solution of D_2O and held there for one hour. After equilibrium was attained in water exchange between the protoplasm and the surrounding water the algal cells were sliced at right angles with a razor blade. During this operation the protoplasmic contents of the cells did not flow out into the surrounding solution. The cut-up cells of alga were kept in the solution for yet another hour so that the external solution could diffuse into the vacuole. At the end of the period of exposure to D_2O the cut cells with the D_2O solution were transferred to a sintered glass filter and sucked dry. During this process the cell walls collapsed and only a mass consisting of parts of the Nitella cells containing the undisrupted protoplasm remained on the filter.

The results of an isotopic analysis of the water found in the cell walls and protoplasm is presented in Table 2.

The data obtained support the contention that the protoplasm and cell walls attain 90% or more of the concentration of the milieu. Probably, the few percent discrepancy between the heavy water in the cell walls and the protoplasm is obtained both because of a dilution of the D_2O by water from the air while the material was being dried on the filter and possibly because of an incomplete diffusion of water from the surrounding solution in contact with one side of the vacuoles of the Nitella cells.

Moreover, collation of the results obtained leads to the conclusion that water in the cell is divided into two parts and that the physiological role of these parts is far different. While water found linked to protoplasmic structure is found to be very mobile, water of the cell sap is on the other hand quite immobile.

TABLE 1. Rate of D_2O Intake by Nitella Cells

Material Analyzed	Replicates	15 min		90 min		25 hr	
		conc. D_2O	water exchanged, %	conc. D_2O	H_2O exchanged, %	conc. D_2O	water exchanged, %
Plant	1	5.92	63	5.84	65	5.48	63
	2	5.66	61	5.38	60	5.47	63
Milieu	–	9.34	100	9.03	100	8.73	100

TABLE 2. D_2O Intake by Crushed Nitella Cells

Expt.	Material Analyzed	Conc. D_2O	% of milieu
1	Cell wall + protoplasm	8.7	95.6
	Water	9.1	100
2	Cell wall + protoplasm	7.1	89.9
	Water	7.9	100

This pattern well explains the results obtained on the influx of heavy water into different parts of the plants when they are transferred from ordinary water to isotopic water, and particularly the data obtained when plants are raised in heavy water [2].

The physiological inequality of the two fractions of water in the cell water also well explains the long-established fact that plants growth begins to slow down even when their water loss is not great [4]. Although in the latter case the total water content of the plant is still at a high level, evidently there occurs a certain lag in its movement into the protoplasm, a movement which is related to the mobility of this water and consequently to change in the metabolic functioning of the plasma proteins.

At the same time it can be surmised that the physiological state of water and the role of cell membranes enveloping the protoplasm are far from similar in the process of water movement in the cell.

Other questions concerning the water regimen of plants could also be considered from the point of view of the physiological inequality of the two forms of water.

SUMMARY

It is shown that when intact Nitella cells are immersed in D_2O from 60 to 65% of the water is replaced, irrespective of the exposure time (15 or 90 min or 25 hours). However, if cells cut up into parts (after removal of the cell sap) are immersed in heavy water, the water exchange connected with the protoplasmic structures attains 90-95% in an hour.

It is concluded on the basis of the experimental results that water bound to the protoplasmic structures is very mobile, whereas that in the cell sap is comparatively stable.

The physiological inequivalence of the two water fractions in the cell satisfactorily explains the long-established fact that plant growth is impeded by even a small loss of water. Although in this latter case the total water content in the plant as a whole is still quite high, uptake of water into the protoplasm apparently slows up somewhat; this affects the mobility of the water and as a result leads to changes in the metabolic functions of the protoplasmic proteins.

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WATER CONDITIONS IN CELL ORGANELLES

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Until recently, investigations of plant water relation have predominantly involved the over-all loss of water by the whole plant or parts of it [1-3]. However, the role played by various tissues and structural elements of the cells in this water loss has not been explored. Therefore, it would be interesting to study the relation between the degree of dehydration of different plant tissues and the water loss of cell organelles and also the ability of separate parts of the plant to exchange water.

Using new methods of investigation we were able to obtain an appreciable insight into various aspects of plant vital activities. By using water containing heavy oxygen Vartapetyan and Kursanov [4] demonstrated the typical features of water flux from the roots into organs of the shoot.

Our paper concerns the problem of partition of heavy-oxygen water in the cell and is an attempt to delineate the localization of water in different cell components: the vacuole, the protoplasmic sap, and also the chloroplasts.

In this study, from the beginning we have tried to consider the cellular organelles, vacuoles and protoplasm not from a cytological but from a physiological point of view according to their functions.

The use of labelled water, H_2O^{18}, permitted us to subject these questions to experimental test.

Because of the O^{18} content of the heavy water the distribution of water in the plant can be determined by analysis with a mass-spectrograph.

The most difficult part of our work was separation of the chloroplasts.

There are many data in the literature concerning chloroplast separation. A number of papers describe this technique [5-11]. In almost all of these papers chloroplast separation was carried out in a 0.4 M hypertonic solution of sucrose or in a phosphate buffer at pH 7. This was done to prevent chloroplast disintegration. In addition, the chloroplasts were separated in a supercentrifuge. This technique was not employed in our work since we had to determine the degree of water saturation of different cell organelles.

We tried various methods for extracting the chloroplasts; these were examined microscopically.* As a result of our studies we concluded that the very best material is obtained by squeezing in a hydraulic press at 150 atm and subsequently centrifuging the pressed sap at 3500 revolutions (about 1000 g). It was essential to keep chloroplasts in the cell sap after the tissue was destroyed. Outside the cell sap, chloroplasts quickly disintegrated.

Vacuolar and protoplasmic sap was separated according to the method of Mason and Phillis [12].

After detailed testing we settled on a method for separation of chloroplasts.

The leaves were dried in a thermostat at 34-38° until 10-20% of the initial water content was lost, and then the leaves were saturated with H_2O^{18}. After dehydration the leaves in this condition were submerged in isotopic water and placed in a chamber containing an atmosphere saturated with isotopic water. The chamber was covered with black paper. After various elapsed times leaves were removed and carefully blotted dry with paper; the leaf halves were then placed in a double-ply linen bag and the lightly colored transparent vacuolar sap extracted with a hydraulic press.

The question arises whether water moving into the intercellular spaces during saturation and when the leaf tips are submerged in water is squeezed out with the vacuolar sap. However, if this were so the leaves would be infiltrated upon resaturation. The absence of indications of infiltration in leaves restored to turgor with H_2O^{18} proves that only vacuolar sap is obtained by this pressing process.

After expression of the vacuolar sap the leaves were quickly pulverized in a mortar, placed in a clean bag as before, and the juice pressed out. The juice obtained was fractionated by centrifugation. The first fraction was obtained at 500-1000 revolutions (300 g) for five minutes in order to free it of the coarse cell fragments. The residue obtained was discarded and the juice was again centrifuged at 3500 revolutions (100 g) for 15 min. The chloroplasts were thrown down in the residue as a layer of slightly oily material. The centrifuge tube containing the chloroplasts was placed in a lyophilization chamber, and freeze-dried under vacuum in liquid nitrogen.

The protoplasmic sap freed of chloroplasts was also transferred to a test tube and placed in the lyophilization chamber. The vacuolar sap was treated the same way.

* We consulted Prof. L. P. Breslavets concerning chloroplast extraction.

TABLE 1. Distribution of Water by Cell Fractions in Phaseolus and Bean

Plant	Cell Fraction	At. % O^{18}	Excess, at. % O^{18}	Conditions
Phaseolus	Chloroplasts	0.258	0.054	Wilted 1.5 hr
	Centrifugate	0.256	0.052	Soaked 1.5 hr
Beans	Chloroplasts	0.368	0.164	Original water
	Centrifugate	0.386	0.184	concn. 1.83 at. % O^{18}

TABLE 2. Distribution of Water by Cell Fractions in Russian Bean

Cell Fraction	At. % O^{18}	Excess, at. % O^{18}	Conditions
Vacuolar sap	0.341	0.137	Original H_2O concn. 1.83 at. % O^{18}
Protoplasmic sap	0.333	0.129	Wilted 1.5 hr
Chloroplasts	0.318	0.114	Soaked 30 min

TABLE 3. Distribution of Water by Cell Fractions in Russian Bean

Cell Fraction	At. % O^{18}	Excess, at. % O^{18}	Conditions
Vacuolar sap	0.45	0.244	Original H_2O concn. 1.83 at. % O^{18}
Protoplasmic sap	0.39	0.186	Wilted 2 hr 10 min
Chloroplasts	0.37	0.166	Soaked 1 hr 10 min

TABLE 4. Distribution of Water by Cell Fractions in Russian Bean

Cell Fraction	At. % O^{18}	Excess, at. % O^{18}	Conditions
Vacuolar sap	0.721	0.517	Original H_2O concn. 2.4 at. % O^{18}
Protoplasmic sap	0.692	0.488	Wilted 1.5 hr
Chloroplasts	0.614	0.410	Soaked 1.5 hr

TABLE 5. Distribution of Water by Cell Fractions in Russian Bean

Cell Fraction	At. % O^{18}	Excess, at. % O^{18}	Conditions
Vacuolar sap	0.765	0.551	Original H_2O concn. 2.4 at. % O^{18}
Protoplasmic juice	0.648	0.444	Wilted 1.5 hr
Chloroplasts	0.650	0.446	Soaked 1.5 hr

About 75 g of fresh leaves was required for each experiment. This quantity of leaves was determined by the fact that it produced enough chloroplasts to yield upon freeze-drying the 1 ml of water needed in mass-spectrographic analysis.

Isotopic analysis of the water was made according to the method of Trofimov† [13-14] in which 1 ml of water is exposed to exchange with 50 mg of K_2CO_3 at 100° C. The exchange was completed in 20 min, after which the carbonate was separated by evaporation of the solution and the carbonate decomposed by pyrophospnoric acid in an evacuated tube.

The exchange produces CO_2 enriched with O^{18}. The isotopic composition is determined by ratio of isotopic peak values obtained on the mass-spectrograph. In this manner the O^{18} content of the CO_2^{18} is determined.

RESULTS

In order to delineate our problem we conducted three preliminary experiments with three crops: oats, Russian beans, and Phaseolus. The beans are a plant which lose water readily and rapidly and wilt with a small water loss. Oats wilt with appreciable water loss. Phaseolus loses water reluctantly.

In these experiments we aimed to separate the chloroplasts without undertaking to fractionate the centrifugate into protoplasmic and vacuolar sap.

It is evident from Table 1 that in Phaseolus there is little difference in the degree of saturation of the centrifugate and the chloroplast with heavy oxygen water, they are in fact nearly the same. In beans this difference is prominent, 0.018 at.%. Apparently this means that Phaseolus is more resistant to dehydration.

A subsequent series of experiments was conducted with beans grown under optimal conditions of water supply and nutrition. Leaves all from the same storey were selected from plants 2-3 weeks old. The experiments were conducted throughout the year with Russian beans. The plants were raised in a greenhouse. Most of the experiments were carried out by soaking for about 1.5 hr although in the beginning we also used shorter periods. At first water enriched with 1.83 at.% of O^{18} was used. Table 2 shows the data obtained with Russian beans. The analysis was done this way during short exposures to this water.

The difference between the degree of saturation of the vacuolar sap and the chloroplasts interested us. As we see, in these experiments the difference between these fractions is 0.023 at.%. There is a 0.015 at.% difference between the protoplasmic sap and the chloroplasts. Since we did not obtain greater differences we decided to lengthen our exposure times. Table 3 presents data for longer exposure times.

It is apparent that the difference in degree of saturation between the chloroplasts and the vacuolar sap reaches 0.080%. The protoplasmic juice and the chloroplasts differ little, 0.020%.

Data from experiments conducted using water more greatly enriched (2.4 at.% O^{18}) are shown in Tables 4 and 5.

In Table 4 the difference between the chloroplasts and the vacuolar sap seems appreciably greater, 0.107 at.% and between the protoplasmic juice and the chloroplasts 0.078 at.%. In Table 5 the difference between the vacuolar sap and the chloroplasts is 0.115 at.%, while no difference was detected between the protoplasmic juice and the chloroplasts.

Having obtained such data we decided to set up still another experiment under a few other conditions. Leaves which had been wilted for 1.5 hr were saturated and placed in a cabinet in an atmosphere saturated with the vapor of isotopic water and left in the dark for 20 hr (Table 6).

Even after such prolonged saturation conditions (Table 6) the difference between the vacuolar sap and the chloroplasts remained, although to a lesser degree (0.6 at.%). The chloroplast concentration of isotopic water coincided with that of the protoplasmic juice. It is possible that under these prolonged saturation conditions an isotopic equilibrium was reached between the chloroplasts, the vacuolar sap, and the protoplasmic juice within the cell.

Complementary experiments were conducted with the radicles of Russian beans in order to clarify the difference in water saturation between protoplasmic juice and vacuolar sap. Five-day-old radicles were put into isotopic water with 2.4 at.% O^{18} for a period of 1.5 hr. The radicles were removed and blotted dry with filter paper. Two zones 2 mm long were sampled from them: the tip, where microscopic examination revealed only meristematic tissue (nonvacuolated), and a second zone 3 mm back from the tip in the clearly vacuolated region of elongation. The material was freeze-dried and the extracted water analyzed for the isotope.

Table 7 shows the data on water flux into meristem and the cells of vacuolated tissue.

It is evident that the difference in degree of saturation of the meristem and vacuolated cells with isotopic water is appreciable. This permits us to conclude that the water reserve of the protoplasm is more stable than the water in the vacuole.

It can be anticipated that further investigations using the isotope method will permit a deeper study of the distribution of water in plant cell components.

Subsequent phases in our work will cast light on the problem as to whether or not chloroplast dehydration is the cause of plant death.

Refinement of technique will be needed for us to carry this work further.

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† B. B. Vartapetyan provided advice during the development of a method for determination of O^{18}.

TABLE 6. Distribution of Water by Cell Fractions in Russian Bean

Cell Fraction	At. % O^{18}	Excess, at. % O^{18}	Conditions
Vacuolar sap	1.09	0.986	Original H_2O concn. 3.04 at. % O^{18}
Protoplasmic juice	1.03	0.826	Wilted 1.5 hr
Chloroplasts	1.03	0.826	Soaked 20 hr

TABLE 7. Distribution of Water by Zones in the Radicle of Russian Beans. Original Water Concentration 2.4 At. % O^{18} Soaked 1.5 hr

Tissue	At. % O^{18}	Excess, at. % O^{18}
Experiment 1		
Meristem	1.42	1.216
Vacuolated cells	1.76	1.556
Experiment 2		
Meristem	1.78	1.576
Vacuolated cells	1.93	1.726

SUMMARY

The distribution of heavy oxygen water in various parts of the bean plant cell was investigated. The O^{18} content in the water of freeze-dried vacuole and protoplasm sap and also of chloroplasts was determined by mass-spectrometric analysis. These fractions were extracted from H_2O^{18}-saturated bean leaves after preliminary dehydration. The vacuole sap was obtained by applying a pressure of 150 atm with a hydraulic press. The leaves were then ground and the protoplasm sap and chloroplasts were separated by centrifugation at 1000 g. In all chloroplast and protoplasm sap fractions, the O^{18} content was smaller than that in the vacuole sap. This indicates that the water reserve in the protoplasm sap and chloroplasts is larger than in the vacuole sap. Evidently water exchange is most rapid in the vacuole sap.

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ABSORPTION OF WATER BY CUT ROOTS

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The numerous investigations dealing with the nature of absorption and exchange of minerals by plant roots have led to the differentiation between passive absorption, via more or less complex diffusion, from active absorption, which is dependent upon the metabolic process and is characterized by the accumulation of elements against concentration gradients.

We deemed it of interest to study the absorption of water from the point of view of division into active and passive processes. We decided on using for this purpose the radioactive method, and selected tritium water for the same. An analogous work was published earlier, where the water was tagged with heavy oxygen [1]. Our results are in good agreement with those obtained by the said authors. We were able to extend their data by showing that the fraction of water which does not exchange rapidly (this water is presumably bound to the cell organulae) is subject to slow exchange with the water of the extraneous medium.

PROCEDURE

Barley seeds, sterilized by means of hydrogen peroxide and thoroughly washed, were germinated on gauze under intensive aeration. Their germination took place in a room at a temperature of 22° C, under 100 percent humidity. During germination the seeds were illuminated with red light. After six days of germination the rootlets were cut off; they were at that time 4-6 cm in length. The rootlets were several times washed with distilled water for a total of approximately one hour, after which they were subdivided into lots of 300-400 mg, and these were placed into small baskets made from plastic-impregnated fabric (Fig 1).

At the start of the experiment, each basket was placed into a plastic test tube (of an approximate volume of 100 ml) containing 50 ml of tritium water aerated by means of air bubbles.

In order to study the effect of 2-4 dinitrophenol (DNP) use was made of tritium water containing 10^{-4} M DNP. In another experimental series with DNP, the rootlets in the baskets were treated with 10^{-4} M DNP for five minutes. After such a treatment the water was rapidly shaken off from the baskets, and they were placed into lucite test tubes containing tritium water with 10^{-4} M DNP. We were thus able to avoid a differential absorption of DNP and of tritium water, which could have distorted the results from the absorption of tritium water only.

The tritium water (approximately 1 C/ml), * which was kindly supplied by SCRGR of the CEA (Saclay, France) was diluted to a final concentration of 0.5 μC/ml. Determinations showed that this radioactivity was actually 0.67 μC/ml.

* As in original – Publisher.

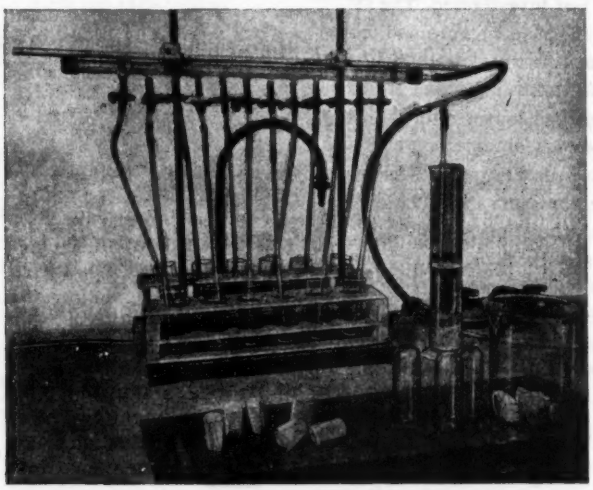

Fig. 1. Setup for the study of water exchange in rootlets of barley by means of the tritium water method.

After an exactly measured period of stay in either the tritium water as such or in tritium water + DNP, the basket was removed and successively washed in four 250-ml beakers of distilled water in order to remove the residual tritium water. For the first series, the rinse was of a 16- to 20-sec duration, and for the last ones less than 10 sec.

After the rinse, the baskets were placed in a lucite test tube containing exactly 50 ml of distilled water through which air was being bubbled. At various intervals a 1-ml sample of this water was taken into a measuring flask and this was further processed for radioactivity measurements. An increase of the latter served as a measure of the desorption of tritium water absorbed by the rootlet.

A few experiments were conducted on the exchange of slowly exchangeable water. The little baskets, containing approximately 400 mg of the rootlets, were placed for various lengths of time (from 2 to 80 min) into aerated tritium water, which either did or did not contain DNP (with preliminary DNP treatment or without it). After rinsing, they were placed for desorption purposes, as in the preceding experiment, for an exactly measured interval of time (15 min) under constant aeration. Subsequently, the little baskets were again rinsed and placed into a lucite test tube containing 50 ml distilled water, and were left there for 24 hours for desorption purposes. Then the radioactivity of the second, and also the first (15 min) desorption water was determined. We were of the opinion that the rapidly exchanging water becomes fully desorbed within 15 min (which our first series of experiments seemed to indicate), and that the water which is desorbed within 24 hr corresponds to the slowly desorbed water, which is bound to cell organulae, for instance.

The weight of the rootlets was also determined after the desorption. They were centrifuged in their little baskets for 1 min at 4000 rpm, and subsequently weighed. We did not carry out a systematic determination of the dry weight of every sample because its exact determination is not essential.

Besides these experiments, there was also conducted a similar treatment of the empty baskets as such for the purpose of determining the correction value caused by incomplete rinsing and the adsorption of tritium water by the basket itself.

The concentration of tritium water was determined by means of the scintillation method. The measurements were conducted at SCRGR, Saclay, by means of a Packard counter. We placed 0.2 ml of the radioactive solution into a special 20-ml ampule, and added to it from a burette 11.2 ml of a solution containing 4g of 2,5-diphenyloxazole, and 0.1 g 1, 4-di (2, 5-phenyloxazole) benzene in 1 liter of redistilled toluene, and finally added to it from a burette 3.6 ml absolute ethyl alcohol. The ampule was shaken up and set in darkness. Afterwards, not later than 12 hr before measurement, the ampule was introduced into the freezer of the scintillation counter (at -10°C). Subsequently, each ampule was placed in the counter itself, and after a time interval of 10 min to bring about a temperature equilibrium, a count of the impulses during a certain time period was taken.

A solution diluted with tritium water served as the control, representing the initial radioactivity for various measurements.

RESULTS

In one of the first experiments approximately 1 g rootlets was placed for 45 minutes in thirty ml of

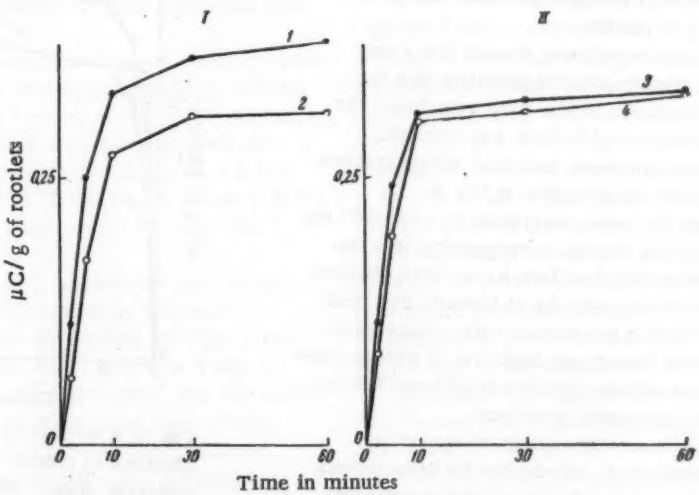

Fig. 2. Curves of exchange of water absorbed by barley rootlets within 30 minutes. I, water; II, water + DNP. Radioactivity of tritium water, 0.5 μC /ml.

TABLE 1. Numerical Values Used in Compiling the Curves of Fig. 3 (duration of desorption 30 min)

Duration of absorption, min	Weight of rootlets, mg	Imp/min in sample	Imp/min after correction	μC/g of rootlets without correction	μC/g with correction
Tritium water					
2	250	23 900	17 400	225	165
5	288	36 300	29 800	300	245
10	245	35 000	28 500	335	275
30	270	40 700	34 200	350	295
6	731	84 700	78 200	275	250
Tritium water + DNP 10^{-4} M					
2	287	25 800	19 300	210	160
5	267	34 700	28 200	305	245
10	302	45 200	38 600	355	300
30	245	37 000	30 500	355	295
6	743	82 200	75 700	260	240

tritium water with a radioactivity of 2 μC/ml. After transfer of the rootlets to plain water the desorption was observed from the first to the sixtieth minute.

Radioactivity determinations gave values within the range of 400,000 to 800,000 imp/min. For such values, the apparatus in use did not provide a proper relationship between the true radioactivity and the one indicated by the apparatus.

In the second experiment, the radioactivity of tritium water, determined by calculation, was equal to 0.5 μC/ml. The absorption by the rootlets lasted for 30 min. The desorption was determined after 2,5,10,30, and 60 minutes. Simultaneously, a series was set up on the absorption of tritium water containing 10^{-4} M DNP. At the end of the experiment the rootlets were weighed, and the following values found, as related to the curves on Fig. 2: Curve 1, 300 mg, Curve 2, 343 mg, Curve 3, 371 mg, and Curve 4, 315 mg. The results in Fig. 2 are calculated per 1 g of rootlets.

Since the second experiment showed that a very large quantity of tritium water did penetrate into the rootlets within 30 minutes, in the third experiment the rate of water penetration with time was studied. The duration of the desorption remained always constant (30 min). The results are presented in Fig. 3.

Each point on the curve corresponds to a 245-302 mg weight of rootlets. The squares, corresponding to a six-minute absorption period in tritium water, were obtained with two 731 and 743 mg samples of rootlets. The final concentration of DNP in the tritium water amounted to 10^{-4} M. Both curves show a very high rate of tritium water penetration. The quantities absorbed by 250 and 750 mg of rootlets are in fairly good agreement.

In this group of three experiments no account was taken of the tritium water adsorbed by the little baskets. This is of minor importance in the second experiment, where the kinetics of desorption of each series were studied on the same basket. One may presume that the difference in curves I and II in Fig. 2 is caused by incomplete drying of the basket following the experiment. In the third experiment the error is larger. The results obtained were corrected on the basis of the corresponding determinations in the latest experiments.

Table 1 represents the figures which were used for the compilation of curves in Fig. 3, as well as the corrected values where the absorption by the basket itself has been taken into consideration; the determinations are those of the last experiments (6500 imp/min).

Fig. 3 shows also the corrected values (dotted lines). Here good agreement can be seen between the 5- and 6-minute absorption of samples of various weight. In addition, one can see from curves 2,3 and 4 of Fig. 2 and

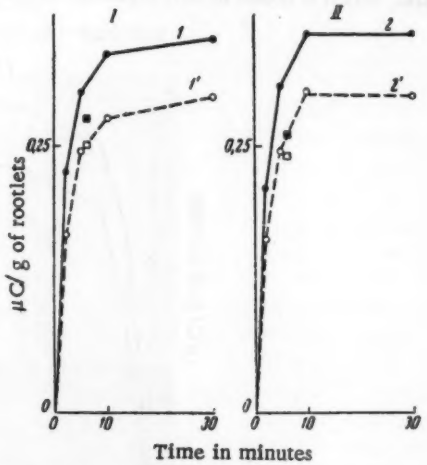

Fig. 3. A 30-minute exchange of water absorbed by rootlets during various time intervals from 20 to 30 min). I, water; II, water + DNP. Radioactivity of tritium water, 0.5 μC/ml.

TABLE 2. Exchange Rate of the Water of Low Exchange Capacity

Duration of absorption, min	Weight of rootlets, mg*	μC exchanged in the sample in 15 min**	μC exchanged 15 minutes later (on 24 hr)	Water exchanged by 1 g of rootlets			
				μC in 15 min (a)	μC 15 min later (on 24 hr) (b)	b/a	total number of exchanged μC
Tritium water							
2	408	1.91	0.030	234	3.7	1.6	238
5	444	2.83	0.080	320	9	2.8	329
10	417	2.83	0.089	340	10.7	3.15	351
20	424	2.83	0.161	335	19	5.7	354
40	443	3.39	0.181	383	20.5	5.2	403
80	429	3.43	0.314	400	36.5	9.1	436
Tritium water + DNP 10^{-4} M							
4	426	2.39	0.049	280	5.7	2.05	286
5	462	3.43	0.072	372	7.8	2.10	380
10	435	3.27	0.144	375	16.6	4.4	392
20	488	3.43	0.174	352	17.9	5.1	370
40	476	3.51	0.194	370	20.5	5.5	390
80	435	3.51	0.304	403	35.0	8.7	438

*Determined after the experiment.

**With correction for water desorption by the basket (desorption of water in 15 minutes, 0.33 μC; on 24 hr, 0.064 μ C).

from corrected curves of Fig. 3, that the amounts of water absorbed after a 30-minute absorption and a 30-minute desorption are almost identical. On the other hand, without correction, the results of the second experiment indicate that the rootlets here absorbed more water than the ones of the first experiment.

In our last experiments another attempt was made to determine whether the water which exchanges with difficulty is capable of a slow exchange. Table 2 gives the results of one of these experiments.

As to the rapidly exchanging water, results were obtained which were analogous to those of the first experiments. The exchange proceeds rapidly, with one half of the total exchange water being absorbed within less than two minutes.

The data on the amount of exchanged water in various experiments are in fairly good agreement: On examining the values obtained after a period of 30 and 40 minutes, one can see that the amount of exchanging water per 1 g of rootlets amounts to 0.615 ml (Fig. 2, curves 2 & 3); 0.64 ml (Fig. 2, curve 4); 0.59 ml (Fig. 3, curves 1' & 2'); 0.57 g (Table 2, tritium water), and 0.55 ml (Table 2, tritium water-DNP).

Dinitrophenol evidently accelerates the exchange. On examining Fig. 2, which represents the result of a 30-minute water absorption by rootlets, and Fig. 3, representing the absorption in time, as well as Table 2, dealing with a more prolonged absorption, one will notice that in the presence of DNP there takes place a rapid stabilization of desorption of radioactivity, while in tritium water as such the absorption and desorption are quite lasting.

Table 2 shows that a 15 minute long desorption is followed by a relatively slower desorption, the magnitude of which depends on the duration of absorption (the error of the supplementary desorption determination is larger when the absorption is of short duration). A comparison of the results obtained for tritium water as such or in the presence of DNP, shows that they are substantially the same. It was possible to conclude from the results obtained from a 40-and 80-min absorption that the accumulation of the slowly exchangeable water is proportional to the time of absorption.

DISCUSSION

Our estimates showed (see Figs. 1 and 2) that after 15-30 min of absorption the water exchange proceeds at high speed during absorption as well as during desorption. We may assume that during the interval of 15 to 30 min the exchange reaches an equilibrium, which according to our calculations amounts to 0.55-0.60 ml of water per 1 g of rootlets. By assuming that 1 g of rootlets contains about 0.9 ml water, it follows that approximately 60-65% of water gets readily and rapidly exchanged with the water of the surrounding media.

These results are in very good agreement with those of an earlier work [1], in which the authors found the renovation, constituting from 50 to 60% of the water of the rootlets, is to a considerable extent constant when the duration of the experiment was from 15 min to 1 day.

Thus, one may assume that the isotope method permits an easy and rapid determination of the amount of free water in the rootlets.

The low exchange capacity water, which represents the bound water of the rootlets, is renovated rather slowly, as can be seen from the results of Table 2. It was found that after 80 min every gram of rootlets is rapid-

ly exchanging 0.60 ml of water, and that from the remaining 0.30 ml, 0.052 ml (or 17%) is subject to a slow exchange. A study of the kinetics of bound water renewal will undoubtedly permit pointing out the various categories of bound water, a problem which we intend to undertake in the near future.

Since the kinetics of water absorption by the rootlets proceed at too rapid a pace, our current method of determination does not permit a high degree of accuracy. Under these conditions the effect of DNP does not stand out with sufficient clarity. However, the course of the curves indicates that in the presence of this inhibitor there takes place some acceleration of exchange phenomena. Besides, the rootlets which were immersed in a DNP solution for ten minutes or longer showed a distinct yellowing phenomenon. It is known that this compound influences the accumulation of stores of energy, which have been liberated during the oxidative processes of the cells.

The acceleration of water exchange in the presence of DNP may indicate that the water retention in the rootlet is not simply a result of diffusion phenomena across membranes, but that a portion of the respiratory energy is being used for the inhibition of water exchange between the rootlet and the surrounding media.

These results may also assist in the interpretation of certain aspects dealing with the absorption of mineral matter.

SUMMARY

It was possible to study the absorption of water by cut barley rootlets by means of water tagged with tritium.

The exchange rate is very high. Within 30 min nearly all of the free water (the readily exchangeable fraction) is exchanged. The bound water is also being exchanged, but at a much slower rate (17% within 80 minutes).

In the presence of dinitrophenol (DNP) the rate of water exchange is accelerated, presumably due to the lifting of some obstacle along the diffusion path.

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PARTICIPATION OF H_2O^{18} IN THE METABOLISM OF PHOTOSYNTHETIC TISSUES

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During studies of the uptake of heavy water by plant tissues, a question arose as to what extent the different cell components (cytoplasm, plastids, etc.) are accessible to water ingress. This question is still interesting because in preliminary work [1-3] it has been shown that when plants were kept in nutrient solution containing heavy water, the plant tissues contained appreciably less heavy water than the external nutrient solution.

Could this not occur because certain cell components (plastids, cytoplasm) are poorly accessible to water entering the plant and also the water they contain is exchanged little with the external water?

It would also be no less important to clarify the extent to which the water entering from outside participates in the cell metabolism.

Unfortunately, here we encounter additional difficulties in technique which interfere with the direct study of H_2O^{18} influx into various components of the plant cell, since the cell must be destroyed prior to separation of its constituent parts. Cell destruction usually leads to mechanical mixing of the water from different parts of the cell. Therefore, we first had to seek other techniques.

As a result, we have selected a method which permits us to obtain certain insights into the uptake of H_2O^{18} from the external nutrient solution by different components of the cell, and also on the participation of this water in biochemical reactions.

This method has chiefly aided research on the isotopic composition of oxygen evolved in photosynthesis [4-6] and the carbon dioxide evolved in plant respiration [7]. In these studies it has been shown that the isotopic composition of oxygen evolved by the cells of green plants during photosynthesis and the oxygen in carbon dioxide from respiration corresponds to the isotopic composition of oxygen in water.

Since the oxygen evolved during photosynthesis originates in the chloroplasts, while the enzymatic system of aerobic cell respiration is centered in the mitochondria

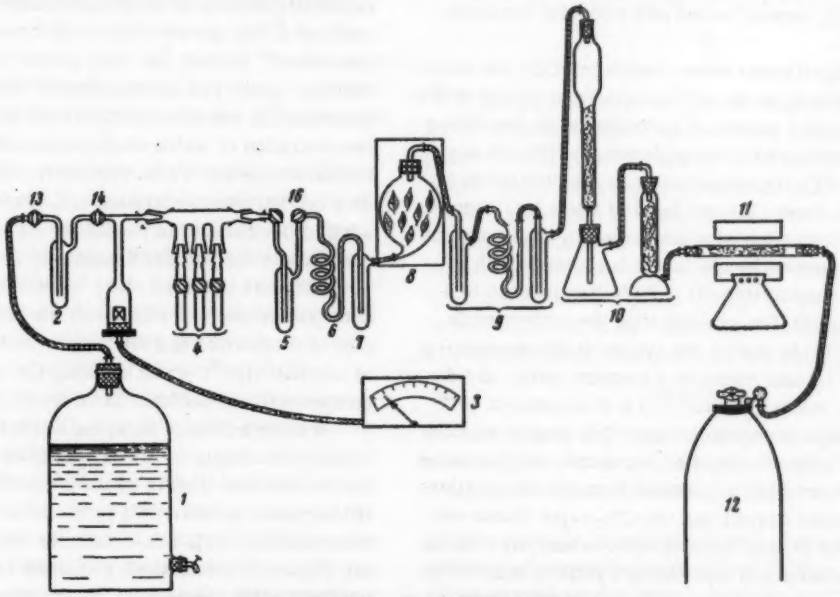


Fig. 1. Diagram of CO_2 collecting apparatus.

and the glycolysis process is carried out in the cytoplasm [8], it would be expected that the isotopic composition of the oxygen evolved during photosynthesis, of the carbon dioxide from respiration, and of the carbon dioxide from fermentation would reflect the isotopic composition of that aqueous medium whence these gases originated. By preparing a suitable form of the plant and then collecting for isotopic analysis the oxygen evolved by photosynthesis and the CO_2 from respiration and fermentation we might obtain insight into the isotopic composition of the oxygen in the water contained in the cellular components mentioned above, in cells not only intact but functioning normally.

Preliminary preparation of the plants consisted in removing the ordinary exchangeable water from the cells and replacing it with heavy water from the external solution. The accessibility of the mentioned cellular components to external water can be detected by isotopic analysis of the oxygen from photosynthesis from the CO_2 of respiration, and the CO_2 of fermentation respectively. At the same time these analyses would show the extent to which the water taken up participates in the chemical reactions of these processes.

Phaseolus vulgaris and the hydrophytes Vallisneria and Riccia were used during the investigation.

In the investigation of the isotopic composition of the oxygen in the CO_2 from respiration and fermentation we used 10-15 day old seedlings of Phaseolus grown in tap water in the greenhouse. Before the experiment the root system of the plant was blotted with filter paper, after which the plants were transferred to Knop nutrient solution containing H_2O^{18}. After a period of 24-40 hours the leaves were removed and the respiration and fermentation CO_2 were collected with a special apparatus (Fig. 1).

In the experiments where respiratory CO_2 was collected, the detached leaves of Phaseolus were placed in the chamber (8) and a stream of atmospheric air was forced through the system with the aspirator (1). The air was first freed of CO_2 impurities using an alkali solution in an absorption tower (10) and freed of water in a liquid-air trap (9). Trap (2) is also submerged in liquid oxygen. The oxygen evolved by the leaves is removed with a solid carbon dioxide trap (6) and (7) then is dried in a liquid-air trap (9). For analysis after the collection is made valve (16) is closed, the system is disconnected at the aspirator (1) and joined to a vacuum pump, and the part between valves (13) and (16) is evacuated to 10^{-3} mm of mercury. A vacuum meter (3) is used to measure the vacuum. After the required vacuum is reached valve (14) is turned, trap (5) is removed from the Dewar flask containing liquid oxygen and the CO_2 vapor frozen out in the ampules (4) and then subjected to analysis with the mass-spectrometer and reported as a ratio of mass 46 to mass 44. After the respiration CO_2 is collected from these leaves the water is removed by lyophilization [9]. The concentration of O^{18} in this water is then determined by equilibrating it with CO_2, which is then likewise analyzed with the mass-spectrometer [10].

In experiments where the anaerobically produced CO_2 is collected, the system is handled the same way except that the plant is kept in an atmosphere of nitrogen gas. Gaseous nitrogen is administered from a tank (12) and traces of oxygen are removed by passing the gas through a layer of glowing hot copper shavings (11). In this case the aspirator (1) is not needed and it is disconnected from the system. The capture and analysis of the fermentation-produced CO_2 and the water in the leaves are conducted in the same way as before.

The hydrophytes Vallisneria and Riccia were used in experiments on uptake of H_2O^{18} by chloroplasts. The plants were totally submerged in Knop nutrient solution containing H_2O^{18}. After an exposure of 2, 4, 24, and 72 hours the oxygen evolved by photosynthesis was collected and analyzed for isotope O^{18} on the mass-spectrometer. The tissue water was analyzed at the same time. The plants were removed from the solution, carefully blotted with filter paper and the water evaporated for subsequent isotopic analysis.

The experimental results are shown in Tables 1 and 2. The figures signify atom percent O^{18} compared to the natural content, assumed here to be 0.21 atom percent.

From Tables 1 and 2 it is apparent that the oxygen in carbon dioxide from respiration and fermentation and the oxygen evolved during photosynthesis is heavily enriched with O^{18}. Consequently, the heavy water from the external solution comparatively quickly reaches the protoplasm and cell organelles and is involved in the metabolic biochemical reactions in them. This is especially evident in those experiments where the oxygen evolved during photosynthesis was investigated. In this case the O^{18} content was even greater than in the intracellular water. The carbon dioxide from respiration and fermentation was also enriched with heavy oxygen, the concentration of which corresponded with its concentration in the leaf water. However, when considering the data on CO_2 from respiration and fermentation caution is advised because of the possibility for isotopic exchange reactions in the carbon dioxide and water.

The data obtained make it possible to conclude that both protoplasm as a whole and the organelles incorporate in themselves and are readily accessible to influx of external H_2O^{18}. Water entering the cell reaches these components and becomes involved in their metabolism.

It is expedient to compose a similar question concerning the origins of the oxygen from photosynthesis in connection with a study of the water metabolism of the chloroplasts. Recently this problem has again come under discussion [11, 12]. The unanimity which prevailed after the first work demonstrating that the oxygen from photosynthesis came from water has wavered because the O^{18} content of oxygen evolved during photosynthesis appears

TABLE 1. O^{18} Content of Respiration and Fermentation CO_2 and Water in Phaseolus Leaves

Product analyzed	O^{18} concentration	O^{18} concentration in external solution
Respiration CO_2	0.75	0.98
Leaf H_2O	0.70	
Fermentation CO_2	0.82	1.18
Leaf H_2O	0.86	

TABLE 2. Isotope Content of Photosynthesis O_2 and Tissue Water

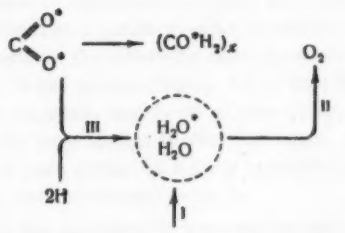
Plant	Product analyzed	Exposure 2 hr	4 hr	24 hr	72 hr	O^{18} concentration in external solution
Vallisneria	Photosynthesis O_2	0.48	0.89	0.87	—	1.01
	Plant tissue H_2O	—	0.71	0.85	—	
Riccia	Photosynthesis O_2	—	—	0.91	0.96	1.00
	Plant tissue H_2O	—	—	—	0.70	

somewhat greater than in the water [4, 6]. Because of this and the fact that the O^{18} content of natural CO_2 is appreciably greater than that of water, certain workers [11] have considered the possibility that the oxygen in carbon dioxide participates along with the oxygen of the water in the formation of the oxygen evolved during photosynthesis.

Not long ago the very careful work of Vinogradov, Kutyurin, Ulubekova, and Zadorozhnyi [12] supported the fact that there is an insignificant excess of O^{18} in CO_2 photosynthesis compared to the O^{18} in water, leading to the conclusion that this non-agreement is due to a selective, preferential absorption of the light isotope O^{16} during the respiration process.

Because of the unusualness of the water metabolism of the chloroplasts we propose here the following point of view for consideration. The water metabolism of the chloroplasts*, which is exceedingly out of the ordinary and substantially different from the water metabolism of any other cell component, is composed of the following basic phases: I, influx of H_2O from the external solution; II, splitting of the water during the process of photosynthesis; III, biosynthesis of water by fixation of one atom of oxygen from CO_2 also during the photosynthesis process. Since the oxygen in CO_2 is 11 μg heavier than the oxygen of water [4] then process III superimposed on process I should lead to an increase in the weight of the water. Since processes II and III occur exceedingly close to one another in the cell an increase in the weight of the water should also be reflected in the isotope composition of the oxygen in photosynthesis phase II.

This might be schematically expressed in the following way†.



Calculations indicate that in order to obtain such an increase in heavy oxygen evolved by photosynthesis (about 1 μg is experimentally detectable) one molecule of water from source III must be photolyzed for each 10 molecules of water from source I.

Keeping in mind the experimental data and the consideration involving the fractionation of oxygen isotopes by photosynthesizing plants during the process of respiration [12], it could be considered that this relationship will be shifted still more toward the use of source I.

SUMMARY

An attempt has been made by employing H_2O^{18} as an indicator to ascertain to what degree the cellular components of photosynthesizing tissues are accessible to

*More correctly, those microzones where the photochemical oxidation-reduction reactions occur.

†The asterisk on the oxygen signifies great enrichment with O^{18}.

external water. The participation of this water in metabolism of the cell (photosynthesis, respiration, fermentation) was also studied.

It is shown that external H_2O^{18} easily reaches the protoplasm and its inclusions, and is rapidly involved in metabolic chemical reactions in them.

On the basis of the peculiarity of water exchange in the chloroplasts, a new viewpoint is proposed to explain the differences in the isotopic composition of photosynthetic oxygen and oxygen of water.

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THE EFFECT OF TRACE ELEMENTS ON THE PROCESS OF GREENING AND ON THE STABILITY OF THE CHLOROPHYLL-PROTEIN-LIPOID COMPLEX

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Numerous investigations have shown that the formation of the leaf's pigment system depends on quantities and relationships, in the nutrient medium, of the elements of mineral nutrition: nitrogen, phosphorus, potassium, and especially magnesium and iron [1-5 and others]. We know that a deficiency in individual trace elements, just as a deficiency in macroelements, causes a different sort of chlorosis. The addition of boron, copper, manganese, zinc, and others to the nutrient medium eliminates the chlorosis symptoms. The data obtained by Bertrand and Andreichena [6] are interesting. They observed in their investigations that there is only one tenth of the zinc and one third of the manganese in etiolated dandelion leaves as there is in green leaves. There are indications that such trace elements as copper, manganese and zinc are concentrated in the chloroplasts [7-9]. These facts make it possible to assay the possible part that trace elements play in the chlorophyll formation processes. However, there is very little work on the study of this question in the literature. Thus, there are indications that copper aids in the accumulation of chlorophyll [10-14] and retards its decomposition in the dark [15] and with aging of the plants [11-13]. With respect to other trace elements, there are only individual indications according to which boron, manganese, zinc and molybdenum aid in increasing the concentration of chlorophyll [14, 16]. The effect of trace elements on the condition of the chlorophyll in the plants' plastids has hardly been investigated.

The study of the effect of boron, copper, manganese, molybdenum and cobalt on the accumulation of chlorophyll in the process of greening, on the resistance of chlorophyll to decomposition in the dark, and on the stability of the chlorophyll-protein-lipoid complex was included in the goal of our investigations.

The experiments were carried out during the summer periods of 1957-1958.

Nutans 187 barley was the object of the investigation in 1957. Seeds of experimental plants were soaked, prior to germination, for a period of eight hours in solutions of trace elements of the following concentrations: 0.2 g/liter H_3BO_3, 0.025 g/liter $CuSO_4$, 0.5 g/liter $MnSO_4$ 0.2 g/liter Na_2MoO_4, and 0.025 g/liter $CoSO_4$. Seeds of control plants were soaked in distilled water. The germinated seeds were placed in a Knop nutrient mixture prepared from double-crystallized salts.

In 1958 the experiments were carried out with nutans Viner barley and "Zolotoi doxhd' " oats. The seeds were soaked in water prior to germination and the trace elements were introduced into the nutrient solution according to the following scheme:

1. Control—Knop + 0.01 mg/liter B + 0.025 mg/liter Mn
2. Mn + Knop + " + 1 mg/liter Mn
3. Cu + Knop + " + 0.025 mg/liter Mn + 0.08 mg/liter Cu
4. Co + Knop + " + 0.25 mg/liter Mn + 0.005 mg/liter Co
5. Mo + Knop + " + 0.25 mg/liter Mn + 0.1 mg/liter Mo

There were ten repetitions of the experiments.

The 1957 experiments consisted of two series. In the first series we traced the effect of trace elements on the accumulation of chlorophyll in greening. Over a period of seven days the plants germinated in the dark and were then transferred into light. The concentration of chlorophyll in the leaves was determined for certain specified periods of time. The measurements were made using the paper chromatography method [17].

The results of these experiments are shown in the figure, from which we see that boron, copper and especially cobalt and molybdenum aid the accumulation of chlorophyll. With this, the effect of the boron and copper is shown with the longer stay of the plants in light.

TABLE 1. The Effect of Trace Elements on the Resistance of Chlorophyll to Decomposition in the Dark (nutans 187 Barley)

Experimental variant	Quantity of chlorophyll in mg/g of green weight			Decomposed chlorophyll in % relative to the corresponding control in light	Quantity of chlorophyll in mg/g of green weight			Decomposed chlorophyll in % relative to the corresponding control in light
	a	b	total		a	b	total	
	Seven days of light				162 hours of dark			
Control	0.58	0.28	0.86		0.24	0.09	0.33	62
Cu	0.64	0.31	0.95		0.33	0.11	0.44	54
Mo	0.71	0.26	0.97		0.30	0.10	0.40	59
Co	0.69	0.31	1.00		0.39	0.12	0.51	49
	66 hours of dark				235 hours of dark			
Control	0.47	0.20	0.67	22	0.17	0.09	0.26	70
Cu	—	—	—	—	0.30	0.10	0.40	58
Mo	0.56	0.20	0.76	22	0.24	0.09	0.33	66
Co	0.62	0.27	0.89	11	0.29	0.10	0.39	61

In the second series of experiments we traced the effect on the decomposition of chlorophyll in the dark. Plants of these experiments were germinated over a period of seven days in the light and then placed in the dark. With a quantitative determination of the chlorophyll we observed that the greatest decomposition of chlorophyll in the dark takes place in the leaves of control plants (Table 1). The lowest percent of decomposed chlorophyll is observed at the beginning for plants whose seeds were soaked in a solution of cobalt sulfate; after 235 hours of darkness the plants with the lowest percent of decomposed chlorophyll were those treated prior to planting with copper sulfate.

The effect of trace elements on the stability of the chlorophyll-protein-lipoid complex was studied on young (seven- to nine-day-old) barley shoots during 1957-1958.

In the first year, preplanting treatment of the seeds was used and in the second the trace elements were introduced into the nutrient solution. The stability of the complex was determined by the Sapozhinkov and Maslova method [18, 19]. The leaves for analysis were boiled for one minute in a phosphate buffer. After this thermal treatment, the chlorophyll was extracted from the leaves with petroleum ether and 96% alcohol. As an indicator of the stability of the complex, we used the quantity of chlorophyll not extracted by the petroleum ether in relation to the total chlorophyll content.

The results of the analysis are given in Table 2. In the cobalt and molybdenum variants both in 1957 and 1958, high stability of the chlorophyll-protein-lipoid complex was observed. In 1958 with the introduction of the trace elements into the nutrient solution, high stability of the complex was observed in the cobalt and molybdenum variants and in the copper variant (Table 2).

In an experiment with oats in 1958, we traced the effect of trace elements on the stability of the chlorophyll-protein-lipoid complex during the various phases of plant development. These data are given in Table 3,

TABLE 2. The Effect of Trace Elements on the Stability of the Chlorophyll-Protein-Lipoid Complex in Barley Leaves (1958 experiments).

Experimental variant	First leaf		Second leaf	
	M	$\frac{M_{on}-M_k}{m_{dif}}$	M	$\frac{M_{on}-M_k}{m_{dif}}$
Control	49		49	
Mn	52	2.0	54	5
Cu	55	4.0	55	6.3
Co	53	2.6	56	7.0
Mo	54	3.3	54	5.0

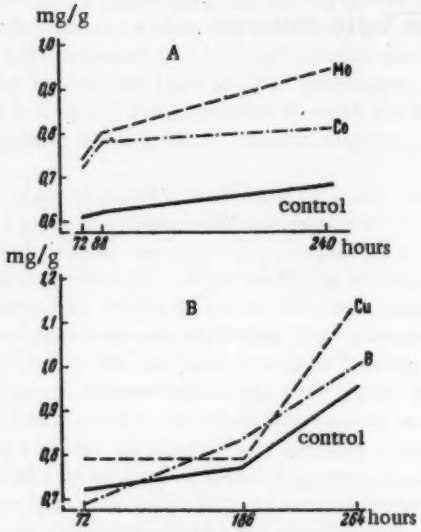
Note: M — chlorophyll not extracted with petroleum ether as % of the total concentration (average of two parallel tests).

from which one can see that copper, cobalt and molybdenum increase the percent of chlorophyll not extracted with petroleum ether in the three-leaf phase and in the phase of stem branching. During the period of tube formation, the highest percent of nonextracted chlorophyll is observed in the control plants. Copper, cobalt and molybdenum decrease the stability of the complex in this phase of development. In the experiment with oats, as in the experiments with barley, an increase in the stability of the chlorophyll and lipoprotein complex under the effect of copper, cobalt and molybdenum is observed during the early phases of development.

A number of investigators [18, 20-22] observed a change in the stability of the chlorophyll-protein-lipoid complex for plants in relation to age and systematic location. Many investigators relate these changes to differences in the protein component of this complex. Osipova [2, 23] showed that physicochemical changes take place in the protein of the chloroplasts in relation to age and nutrient conditions. In experiments in vitro [24, 25] it was shown that the quantity of chlorophyll

TABLE 3. The Effect of Trace Elements on the Stability of the Chlorophyll-Protein-Lipoid Complex in Oat Leaves

Experimental variant	Third leaf		Third and fourth leaves				Fifth leaf			
	three leaf phase		beginning of stem branching phase		stem branching phase		tube formation phase			
	M	$\frac{M_{on}-M_k}{m_{dif}}$	M	$\frac{M_{on}-M_k}{m_{dif}}$	M	$\frac{M_{on}-M_k}{m_{dif}}$	M	$\frac{M_{on}-M_k}{m_{dif}}$	M	$\frac{M_{on}-M_k}{m_{dif}}$
Control	65	—	67		49		61		66	
Cu	—	—	73	3.9	54	2.2	53	4.3	55	6.7
Co	70	2.78	73	3.9	62	5.7	54	3.7	53	7.9
Mo	71	3.3	74	4.5	59	4.4	46	8.8	59	4.3



The effect of cobalt and molybdenum (A) and boron and copper (B) on the accumulation of chlorophyll in barley leaves. Vertical: Concentration of chlorophyll during the time the plants are in light in mg/g of green substance; horizontal: dark.

connected with the protein depends on the chemical composition of the protein and that various amino acids are connected with the chlorophyll in different quantitative relationships.

From the data in the literature on the great role of trace elements in the protein metabolism of plants [26-30] we are inclined to explain the effect of trace elements on the stability of the chlorophyll-protein-lipoid complex by their action on the protein part of this complex.

SUMMARY

1. The trace elements boron, copper, cobalt and molybdenum aid in the accumulation of chlorophyll in barley leaves.

2. Cobalt, molybdenum and especially copper retard the decomposition of chlorophyll in the dark.

3. Cobalt and molybdenum increase the stability of the chlorophyll-protein-lipoid complex in the leaves of young barley shoots and during the early phases of development of oats (up to the tube-formation phase).

The work was completed under the direction of M. Ya. Shkol'nik, Director of Biological Sciences, to whom the authors extend their thanks.

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*See English translation.

THE NATURE OF PLANT FROST RESISTANCE

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The entire territory of the Far North is covered by vegetation which becomes totally frozen in repeated spring and autumn night frosts and quickly revives in the morning thaws; the same thing occurs to the various flora of high-mountain zones; many such examples are also found in moderate latitudes. The investigator's problem is to detect those functions by which the plant resists freezing and is restored to normal viability on thawing.

The study of nature's mechanism for sudden revival of frozen plants was begun with experiments on the whole plant by allowing it the opportunity to mobilize all of its adaptive possibilities and permitting no artificial interference with the normal course of its water supply, transfer of substances and respiration. The chlorophyll-bearing tissues of the leaf were treated by freezing the whole plant (of different species) in a refrigerator at -3 or -5° and then thawing in the laboratory. Samples were promptly cut from the labelled leaf and were immediately infiltrated by solutions of iodine in potassium iodide; subsequently the cuts made by hand were examined microscopically. Iodine fixates the material well and aids in exposing the structures of plastids, cytoplasm and nucleus. Before freezing the plastids were poorly tinted by iodine and contained single small starch grains. The cell content in a frozen leaf appeared as a destroyed agglutinate intensely stained reddish-brown by iodine with an abundant inclusion of large starch grains. After thawing, when the plant took on a perfectly normal appearance (dullness of leaf, turgor), brightly green chloroplasts appeared anew, almost unstainable by iodine and totally starch free. Differing from the original ones, the plastids now were welded by a thin yellowish film with a multitude of apertures.

A detailed study of the dynamics of intracellular changes in freezing and thawing of the plant warranted the schematic structure depicted in Fig. 1. In Stage 1, before freezing, the plastids are normal. On freezing of the leaf the cell contents are agglutinated and destroyed; as a rule, intense starch granule formation is observed (Stage 2).

The initial seconds of cell thawing are accompanied by a division of the coagulum and its transmission to the cell poles or its accumulation at one end (usually the lower one); the central cell portion is occupied by a colorless irregular cytoplasmic small cylinder (Stage 3). Subsequent stages (4-8) of thawing in frost-resistant plants (cabbage, wild potato) are completed in a few minutes (5-10); in less resistant plants they are prolonged up to 30-50 minutes (in cultivated potato). During this period a new formation of plastids at the expense of the plasmic cylinder and coagulum occurs in the cell: Stage 4, ruptures appear in the plasma in the form of small apertures arranged in circles; the coagulum begins to resolve, starch disappears; Stage 5, the cytoplasm acquires the form of a filmy cap paving the cell cavity; areas in circular form outlined by apertures are tinted bright green by chlorophyll. Stage 6, the new plastid circles are thickened, take on clear outlines; the apertures dividing the plastids are widened; coagulum almost disappears; starch is absent. Stage 7, new plastids are connected only by single braces (remains of the plasmic cap); coagulum disappears. Stage 8, the braces are resolved, isolated chloroplasts are normal.

New formations of plastids (Stages 3-8) are indispensable conditions of leaf survival on thawing: If the cells perish, the destroyed agglutinate with the enclosed starch becomes stabilized.

The process of chloroplast destruction at the moment of freezing evidently has several preliminary stages, the sequence of which still needs accurate definition. Photomicrographs in Fig. 2 depict palisade cells of wild potato Sol. schreiteri leaves at different stages of freezing and thawing.

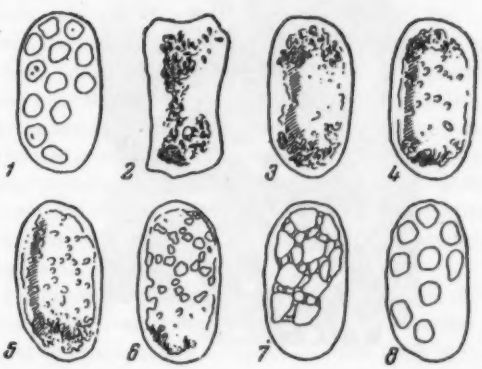

Fig. 1. Dynamics of intracellular changes in freezing and thawing of plants (explanations in the text).

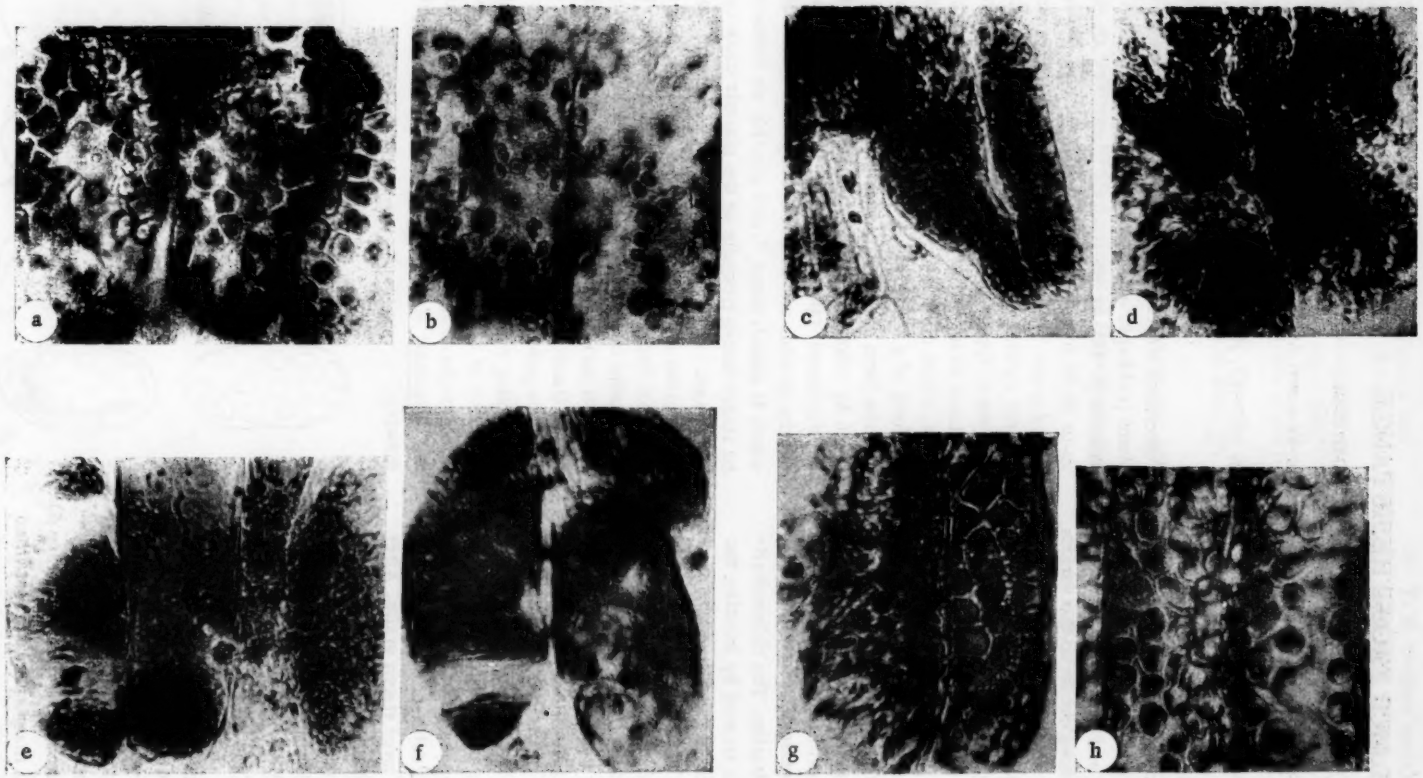

Fig. 2. Cell photomicrographs of the leaf's palisade parenchyme during freezing and thawing of the whole plant of wild potatoes. a) Before freezing; normal plastids with starch granules. b-d) In a frozen state: b) the plastids are compressed, they strongly refract light, they manifest a thickened ring-shaped film (compression stage); c) the ring-shaped film is broken into sickle-shaped segments; the central portion of plastids combines with the cytoplasm's granular mass (segmentation stage); d) chloroplast destruction and the appearance of numerous starch granules (swelling stage). e-h) Thawing period: e) in warmth 3-5 minutes, agglutinate of old plastids at the cell's terminals; in the center a cytoplasmic cylinder with apertures (materials for new plastids); the cell on the right is dead, chloroplasts and starch destroyed; f) the same period; another type of cytoplasmic small cylinder in the same leaf; g) in warmth 18 minutes, new plastids are very clearly defined, but are still welded by braces—remnants of the cylinder; h) in warmth for 35 minutes; normal but still welded chloroplasts.

There are reasons for speculating that the nucleus also participates in the described reconstruction; before freezing (Fig. 2a) it is manifested clearly; in the compression stage (Fig. 2b) its features are indistinct, as in lysing nuclei; in the subsequent stages the nucleus could not be observed, but there appeared a circular refractive body resembling an expanded nucleus (Fig. 2f); a normal nucleus is visible only at the time of new plastid formation. Aleksandron and Savchenko [1] observed the disappearance of the nucleus in winter and its appearance in spring. However, proof is necessary that the nucleus is actually lysed, and not merely disguised by the agglutinated mass.

The starch granules are preserved intact in cells killed by frost, the chloroplast remains destroyed, and frequently there remains the earlier-described light refracting droplike formation.

In field conditions the freezing and revival of chickweed leaves were accompanied by the same changes in intracellular structures; a similar picture was observed in cultivated potatoes, equine sorrel, camomile and others. Cabbage subjected to many days of freezing in October at -8 , -11° immediately began a new formation of plastids (Fig. 3) when brought into the laboratory (with a frozen lump of soil).

To check the order of changes found in whole plants, experiments were conducted on freezing and thawing of leaf sections. Two methods were used.

1. A leaf section was frozen in a drop of water directly under the microscope at -4 , -6° with the use of vital dyes (to prevent overfreezing a cotton wick was placed in the preparation). In the experiment with camomile leaf (stained by neutral red) at the beginning of refrigeration the plastids compressed and became strongly refractory. A few seconds after ice crystals formed the cell content immediately curled up, transforming it into a structureless green mass. At this moment the dye dissolved diffusely in the vacuole, separating in the form of drops and nodules. According to accepted concepts, such a separation of neutral red is incompatible with the death of cells; we did not observe any transfer of stain to the cytoplasm or nucleus. Upon very slow thawing for 1-1.5 hours, a process of new plastid formation from the cytoplasmic cap and coagulum occured in some single cells. At the cap stage the dye again was distributed diffusely in the vacuole, which characterizes the high viability of the cells.

2. A series of adjacent sections were prepared from the leaf, no more distant from each other than 1-2 mm for purposes of close comparison. The groups of sections (except the initial one) in preparations with a drop of water were frozed at -3 , -5°. This method permits a study of dynamics of changes with an immediate fixation in timing and the use of a wide range of postvital dyes. In Fig. 4 photomicrographs are shown of camomile leaf cells fixated by iodine.

Every cytomorphological conversion is related to corresponding biochemical changes. The difficulty of detecting the latter is that the method of average sampling by which ordinary biochemistry operates is inapplicable for investigations of this type: Conversions occur so fast and the difference in quality of individual leaves and, at times, of individual tissues is so great that the least disruption in timing or a minute's thawing in collecting the batch bring about a distortion and "blurring" of results. The range of individual tissues is noted especially frequently in periods of repeated frosts when a portion of internal tissues (mostly the spongy parenchyme) is irreversibly damaged, while the external appearance of the plant is normal. It is clear that in such cases the cut-outs throught the thickness of the leaf characterize the "average sample" between live and dead tissues. This statement proves the need of strictly synchronized cytochemical control in the study of metabolic reactions.

By bringing the samples to a minimum (20-25 mg) and cutting out disks by stages from the same leaf, we succeeded in detecting changes in aminoacids by the ninhydrin reaction. The intensity of color reaction is shown in the Table by a 5-integer scale.

The accumulation of aminoacids on freezing the leaf denotes the splitting of proteins at this stage. The new formation of plastids on thawing is accompanied by a sharp decrease in the quantity of aminoacids, i.e., in protein synthesis. In cases when the leaf dies no decrease in aminoacids on thawing is observed.

The data obtained denote deep functional-morphological changes within the cell, securing its revival on thawing. Evidently freezing does not actually cause cessation of vital functions; disintegration of protein occurs in the cold, furnishing substances for its subsequent resynthesis with formation of new plastids; the forming starch evidently serves as the most compact and speedily mobilized form of energy reserve for protein synthesis. It is assumed that the cause of death of the leaves depends on the irreversible suppression of some link in the chain of preliminary processes operating in the cold, while the plant dies during thawing if the necessary regenerating reconstruction cannot be realized.

Most authors consider the harmful effect of plasma protein coagulation on freezing of tissues indisputable; the protective function of soluble sugars in the cell sap is expressed, according to the prevalent theory, in preventing proteins from coagulation. Schaffnit and Wilhelm [13] consider the value of partial protein decomposition in the cold is its transition to a more resistant noncoagulating form.

Permit us to state our point of view, based on observation. The conversion by a "jerk" of well-formed cellular structures into a compact formless lump intensely colored by iodine denotes the presence of coagulation in some portion of the protein. The fact of subsequent new formation of plastids (and evidently the renativation of protein) shows that in this case the

braces remnants of the cylinder; h) in warmth for 35 minutes; normal but still welded chloroplasts.

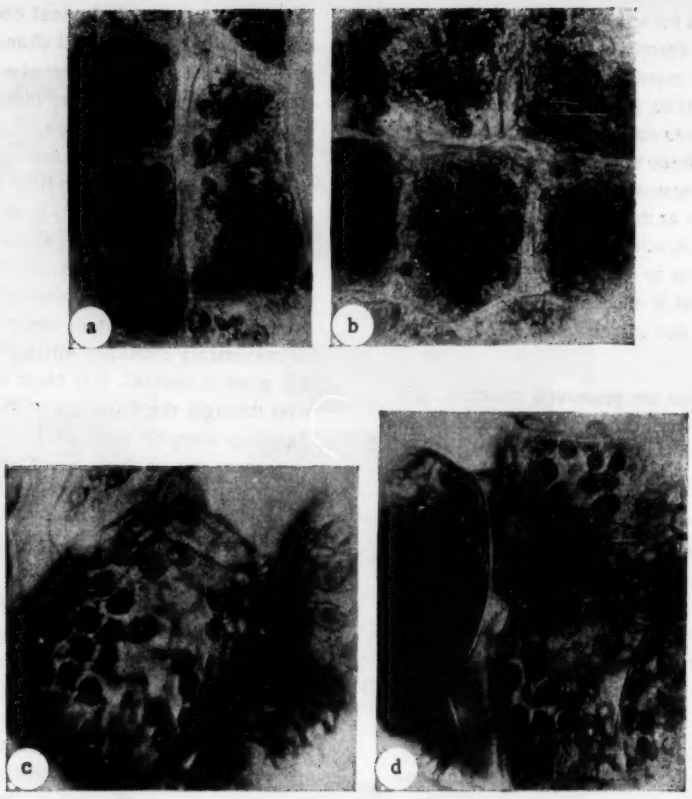

Fig. 3. Photomicrographs of cabbage leaf cells after laboratory thawing of the field-frozen whole plant. a and b) in a frozen state: a) segmentation stage; b) destruction and swelling stage, abundant starch; c) 4 minutes in warmth, new plastid formation; d) 9 minutes in warmth, new plastids; the nucleus is visible.

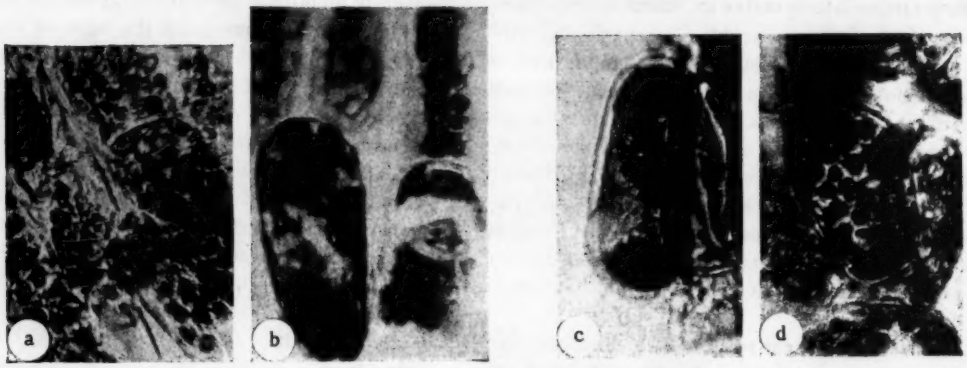

Fig. 4. Photomicrographs of camomile leaf cells fixated by iodine. a) before freezing—normal isolated plastids; b) section is frozen —the agglutinated chloroplast spreads to the cell ends; c) 36 minutes in warmth; cytoplasmic cap stage; agglutinate at cell poles is intensely colored brown by iodine; the cap is nearly colorless, pale yellowish, but spaces between apertures are bright green (in the photograph they are dark in the cell's center); d) 40 minutes later in warmth; chloroplasts are still fused, but plastids have already formed.

Intensity of Ninhydrin Reaction for Aminoacids on Freezing and Thawing of Leaf

State of plant	Leaves revived on thawing				Leaf died
	cabbage	camomile	equine sorrel	potato selected variety	
Before refrigeration	2	2	1	2	1
Beginning of refrigeration	4	–	3	2	–
Frozen	5	5	5	4	5
Thawed	1	1	1	1	5

coagulation was by no means fatal to the plant. The literature is rich in examples of reversion not only of "disguised" denaturing phenomena [2, 10, 11], but also of coagulation and congealing of proteins [14]. It can only be assumed that in the future it will be definitely proven that processes of reversible denaturation appear as natural and necessary links in the complex chain of adaptive functions.

The destruction of the plastid apparatus in the cold and its re-formation on thawing has been established by many authors in wintering plants, in leaves of evergreens and in the cells of bark periderm of woody plants [1, 3-9, 12, 15]. In this we see an important proof of the community of adaptive reconstruction of plants in frosts of short duration, and also in wintering.

SUMMARY

1. An adaptive system was studied which permits Northern plants to become viable after deep freezing. Intracellular changes were detected common to representatives of various gramineous plants. Upon freezing a destruction of chloroplasts, splitting of proteins and starch synthesis occur in the leaf cells. Upon thawing new chloroplasts are formed from the agglutinate of old plastids and cytoplasm.

2. The indicated functional morphological reconstruction is identical in different plants irrespective of the degree of their frost resistance but this reconstruction in plants with greater resistance can be accomplished upon deeper freezing and more rapidly than in non-resistant forms.

3. There is no doubt that this data is far from an exhaustive study of the total complexity of adaptive reconstruction: We are simply stating some facts that might be used as a basis for techniques and approach for further investigation, the application of which should prove fruitful.

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*See English translation.

PROTOPLASMIC VISCOSITY OF LEAVES OF SOME THERMOPHILIC PLANTS RENDERED COLD RESISTANT BY APPLICATION OF VARIABLE TEMPERATURES

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The method of influencing plant seeds by means of low temperature was first used in 1870 by a Petersburg gardener, Grachev [1], who in this way succeeded in obtaining a shortening of the vegetative period and the ripening of corn under conditions of a short northern summer. Using this method, many investigators [2, 3] succeeded in increasing cold resistance and accelerating growth and development, as well as increasing the yield of such thermophilic plants as cucumbers and tomatoes.

Recently, a new method of hardening seed of thermophilic plants by the application of variable temperatures was introduced; this attracted the attention of several investigators [4-11 and others]. Many of them compared the effectiveness of both of these methods—uninterrupted and interrupted cooling—and concluded that uninterrupted cooling was less effective than seed treatment with variable temperatures.

Experiments concerned with treating seeds with variable temperatures gave a positive effect under various soil-climatic conditions; in the Moldavian, Moscow, and Penzensky Provinces, in the Trans-Ural and Altai region, and even in the Polar region.

Acknowledging the unconditional effectiveness of the method in question, many of the authors [3, 12, and others] emphasized that the object of future experiments should be the establishing of the exact conditions of hardening for various soil climatic conditions of the Soviet Union, accounting for the specific reaction of various varieties of plants to hardening. The first steps in this direction have been made in the investigations of Shutov and Belyaev [3], Kodymskii [13] and others.

Together with the studies of an agronomic nature, physiological studies have recently appeared [2,3,6,7, 10,14] disclosing the specific characteristics of metabolism which determine the increased cold resistance and great yield of plants rendered cold resistant by variable temperatures before their sowing. The results of these experiments permit us to conclude that cold resistance of plants depends on the specific features of metabolism of the entire plant. Cold-resistant plants when cooled progressively changed their metabolism, i.e., their reaction to a severe change in external conditions was of a more active adapting character. The specific characteristics of basic metabolism are closely correlated with the dynamics of the colloidochemical phenomena in the protoplasm. That is why investigators engaged in the study of the resistance of plants to various unfavorable factors of the external enviroment attach great significance to the colloidochemical properties of protoplasm: Its viscosity is a characteristic indicator of these properties.

Our experiments were performed from 1956 to 1957 in the Dolmatov, Kurganskaya Province on the fields of the Chkalov kolkhoz.

The climate of this part of the Trans-Urals is distinguished by its extremely continental nature. Spring is characterized by cold and strong winds, severe changes in temperature during the course of the entire day, and frequent frosts which reach -6° even during the first ten-day period of June. Sometimes frosts occur within the second and third ten-day period of June. The frost-free period is not extensive—in some years only 65 days. Fall frosts begin early—frequently at the end of August. If one were to characterize briefly the weather conditions in 1956 as especially cold and dry, then 1957 could be characterized as hot and dry during the first half of the summer with extremely heavy precipitation during the second half of the summer. Since warm temperature is an especially limiting factor for the cultivation of crops of tomatoes, cucumbers, and corn, which we studied under conditions of Western Siberia, then, as a whole, one can consider the hot year of 1957 as more favorable for their growth than the cold and dry year 1956. The contrast of both vegetative periods, in respect to temperature, provided favorable conditions for comparing the effectiveness of the method studied under various weather conditions.

The seeds were hardened in the following manner: The seeds were soaked in water (corn — for 48 hours, cucumbers and tomatoes—for 12 hours). The swollen seeds were exposed to the influence of the variable temperatures (12 hours of heat—from 18 to 20° - and 12 hours of cold—from 0 to -3 or -5°). The seeds were chilled in special cold chambers. Depending on the need, the seeds being hardened were watered and turned daily. Dry seeds served as a control (dry control), as well as

soaked seeds—48 hours for corn and 12 hours for cucumber (wet control). A wet control was lacking for tomato.

We were also interested in the question of the reaction of various varieties of different origin to hardening; therefore we chose three varieties of corn for study: Pervomaiskaya—sowing from local seed, medium ripening variety; Saratovskaya 2— seeds from Saratovskaya reproduction, late ripening variety; Uspekh—seeds from Krasnodarsk reproduction, late ripening variety. Two varieties of Talalikhin tomatoes were chosen—seeds of Sverdlovsk reproduction, early ripening variety and Gruntovyi Gribovsk 01180—seeds from Gribovsk vegetable experiment station near Moscow, late ripening variety. Cucumbers were represented by the variety Nerosimye—seeds from Gribovsk experiment station.

The design of our experiments was as follows: 1) tomatoes—control (dry) and hardened 5, 15, 25, 30 days; 2) cucumbers—control (dry) and hardened 18 days; 3) corn — control (dry) and hardened 5, 15, 25 days. Variety Saratovskaya 2 had another variant—wet control. During the course of the individual stages of a plant's development significant changes occur in several of its properties. The change in protoplasmic viscosity in relation to the growth of the plants and their organs was clarified by the work of many investigators [6, 15, 16 and others] who did not obtain corresponding results. Maksimov and Mozhaeva [17] considered one of the causes of this variation to be the diversity in the objects and methods of the studies, and pointed out the need for a more systematic study of this problem.

In connection with this we therefore studied the change in the ontogeny of protoplasmic viscosity in control and hardened thermophilic plants.

Protoplasmic viscosity was determined by the plasmolytic method [18] during plasmolysis, i.e., during the change from spasmodic or concave plasmolysis to convex. In connection with this, since the protoplasmic viscosity in the plants which we studied reaches high values, and as our observations showed, the diversity in the isotopic concentration of the plants from the different variants of the experiment is not great, and the diversity in protoplasmic viscosity is considerable between the variants, therefore in order to facilitate the investigation we studied the protoplasmic viscosity of a one molar sucrose solution. The viscosity was determined in the cells of the upper epidermis of the leaf.

Our data showed that the change in protoplasmic viscosity during ontogenesis was completely regular in nature, for example: In control and hardened plants viscosity increased from the moment sprouts occurred, then it dropped during the flowering period, and then rose again during the fruiting period. But it appeared that hardening does not always result in an increase of protoplasmic viscosity during the early stages of ontogenesis. The picture analogous to data obtained by Genkel' and his co-workers was observed for corn variety Pervomaiskaya, tomato variety Gruntovyi and cucumber variety Nerosimye. In these plants the viscosity of the protoplasm in the hardened plants during the first phases of growth was higher than in the controls; during reproduction an extremely sharp decrease in viscosity was observed in the hardened plants (Fig. 1). In corn variety Uspekh during the first stages of development the rule was as follows: Hardened plants exhibited greater protoplasmic viscosity, but during the period when reproductive organs were formed the viscosity in the

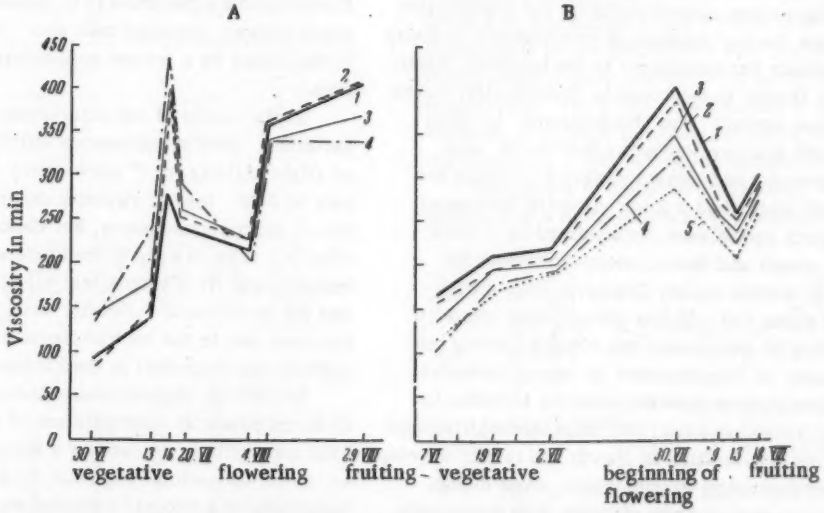

Fig. 1. Protoplasmic viscosity of corn leaves variety Pervomaiskaya (A) and variety Saratovskaya (B) during the vegetative period following various influences on the seed.

TABLE 1. Protoplasmic Viscosity (in min) of Corn Leaves Variety Pervomaiskaya

Date of determination	Phenophase	Leaf from bottom	Hardening (in days)	Not chilled	Chilled
June 19	Vegetative	5	Control	270	315
			5	280	270
			15	290	275
			25	300	260
July 30	Mass flowering	10	Control	245	235
			5	200	190
			15	200	140
			25	215	175

control plants dropped especially low. In corn variety Saratovskaya 2 and tomato variety Talalikhin hardening generally did not result in an increase of viscosity: The viscosity of protoplasm in the hardened plants was lower than the viscosity of the protoplasm in the controls throughout the entire vegetative period (Fig. 1B).

During the fruiting period the protoplasmic viscosity increased in the controls and in the hardened plants, but in the hardened plants the viscosity did not increase as significantly; this apparently indicates the presence of more active form-controlling processes during this period.

Usually the decrease in protoplasmic viscosity facilitates the increase in rate of growth and development of the plants [19]. However, as the results of our experiments show, plants possessing a lower protoplasmic viscosity are not always characterized by more intensive growth and development. Our data concerning the dynamics of protoplasmic viscosity in corn variety Pervomaiskaya (1956 experiment) and Uspekh indicate a positive correlation between low protoplasmic viscosity and a high rate of growth and development. During the first stages of ontogenesis the hardened plants (having a greater protoplasmic viscosity) lagged in growth compared with the controls (having a lower viscosity). During the reproduction period the more marked decrease in protoplasmic viscosity occurred in variety Pervomaiskaya in the hardened plants, and in variety Uspekh in the controls. Specifically, during this period these variants were characterized by more intensive growth and development. Likewise, in corn variety Saratovskaya and tomato variety Talalikhin the hardened plants, which had a lower viscosity throughout the entire growth period, were distinguished by a more rapid rate of growth and development throughout the entire time. In tomato variety Gruntovyi even though the hardened plants had a higher protoplasmic viscosity at the beginning of growth than the controls, during this period they were all characterized by more intensive growth and development than the controls. Likewise in corn variety Pervomaiskaya (1957 experiment), hardened plants exhibited acceleration in growth and rate of development from the first stages of ontogenesis, even though they had a higher protoplasmic viscosity than the controls.

Genkel' and Margolina [15, 20] showed in their experiments that there is a relation between cold resistance of plants and the magnitude of protoplasmic viscosity. The authors concluded that all measures which decrease the protoplasmic viscosity will also facilitate a greater resistance to frost.

Enileev [21] and Anan'ina [19] in their investigations showed that ammonium silicate has the ability to decrease protoplasmic viscosity in plants and at the same time increase the resistance of plants to low positive temperatures.

Genkel' and Badanova [22], and Badanova [23] studied the influence of cations from mineral salts and anions of organic salts, as well as various forms of nitrogenous nutrients, on the magnitude of protoplasmic viscosity on heat and cold resistance of plants. The data from their experiments indicate that those factors which decrease protoplasmic viscosity at the same time induce an increase in cold resistance of plants.

We made some observations on the changes in protoplasmic viscosity of the control and hardened corn and tomato plants under the influence of low temperatures. Discs cut from the leaves of control or hardened plants were chilled in moist atmosphere in petri dishes. All of the experiments were done in replicates of five. As Kushnirenko's experiment [14] performed on these same plants showed, hardened corn and tomato plants are distinguished by a greater cold resistance than the controls.

As the results of our experiments showed, control and hardened corn plants reacted differently to chilling. At slight chilling (to 3° above zero) there was no injury to corn leaves; viscosity decreased in the control and in the hardened plants, but viscosity decreased considerably more sharply in the hardened plants. At lower temperatures (to 1°) some leaf injury was usually induced, and the protoplasmic viscosity changed differently in the hardened and in the control plants: It increased in the controls and decreased in the hardened one (Table 1).

In 1957 we chilled tomato leaves in different stages of development at a temperature of 1° for five hours. In 1958 the chilling was done at a temperature interval of +4 to -3° for various lengths of time. Tomatoes, being indigenous to a tropical climate, are distinguished by a greater need for warmth. At a temperature close to 0° and with an abundance of moisture, tomatoes became

TABLE 2. Protoplasmic Viscosity (in min) of Tomato Leaves Variety Gruntovyi

Phenophase	Experimental variants	Leaf	Date of determination	Not chilled	Chilled at variable temperatures and different lengths of time					
					+4°.4 hr	−1°.4 hr	+4°.24 hr	0°.24 hr	−2°.24 hr	— — — −1°.40 hr
Vegetative	Control	3	2. VII	140	135	−1°.4 hr	−1°.8 hr	−1°.18 hr	−1°.24 hr	90
	Chilled			160	145	95	85	80	70	80
Mass budding	Control	6	17—18. VII	100	80	−2°.4 hr	−2°.8 hr	−2°.18 hr	−2°.24 hr	−2°.40 hr
	Chilled			115	90	75	70	70	65	135
End of budding	Control	7	23. VII	100	75	−3°.4 hr	−3°.7 hr	−3°.16 hr	−3°.19 hr	120 −3°.21 hr
	Chilled			110	80	70	75	70	40	Death of leaves
End of flowering	Control	9	10. VII	95	80	−3°.4 hr	−3°.7 hr	−3°.16 hr	−3°.19 hr	105 −3°.21 hr
	Chilled			85	70	60	50	50	95	—
Mass fruiting	Control	19—11	26. VIII	140	115	−3°.4 hr	−3°.7 hr	−3°.16 hr	−3°.19 hr	Death of leaves
	Chilled			120	80	— —	— —	110	125	

TABLE 3. Cold Resistance of Tomato Leaves Variety Gruntovyi (Percentage of live cells in the visible field of the microscope after freezing)

Experimental variants	Budding	Flowering	Fruiting	
	−4°.4 hr	−3°.21 hr	−3°.17 hr	−3°.19 hr
Control	2	9,4	6	0
Chilled	12	60,0	47	10

diseased, whereas at freezing temperatures of -0.5° or -0.8° they died. We worked with a specially developed frost-resistant tomato Gruntovyi Gribovsk . 01180, which can withstand temperatures of -2 to -3° without injury, and Talalikhin which readily endures a temperature close to 0°.

Our observations confirmed the data obtained by Zholkevich [24], and Genkel' and co-workers [7], which showed that a regular decrease in protoplasmic viscosity followed by its increase is observed in relation to the duration of chilling, whereupon, as our results showed, in hardened, more cold-resistant plants protoplasmic viscosity decreases considerably more than in the controls (Fig. 2).

Lower temperatures and their prolonged effect induced a sharper decrease in protoplasmic viscosity. At very long exposures to chilling protoplasmic viscosity increased first in the control plants, and then upon a longer exposure to chilling also in the hardened plants. This increase in protoplasmic viscosity occurs not only upon the death of the plants in relation to coagulation of the protoplasm, but also occurs when the plants are injured by low temperatures (Table 2), whereupon coagulation of the protoplasm does not occur since the leaves remain alive and turgid after the chilling for several days. Therefore, this increase in protoplasmic viscosity is of great significance during the death of thermophilic plants due to low temperatures.

The results of our observations bear witness to the presence of a more resistant protoplasmic colloidal

Fig. 2. Protoplasmic viscosity of tomato leaves variety Gruntovyi after variable exposures to -2°. 1) Control; 2) hardening.

system in hardened plants since protoplasmic coagulation occured at longer exposures to chilling in the hardened plants than it did in the controls (Table 2).

We also determined the cold resistance of tomato variety Gruntovyi; these determinations confirmed Kushnirenko's data [14] concerning the greater cold resistance of hardened plants (Table 3).

Our observations also confirmed the significance of lowered protoplasmic viscosity for cold resistance of plants.

Cold resistance of plants changes during ontogenesis in relation to changes in the environment and is closely correlated with the colloidochemical properties of the protoplasm, especially with its viscosity. During the flowering period, when protoplasmic viscosity declined sharply, leaves from tomato variety Gruntovyi are especially cold resistant (Tables 2,3). Upon transformation to the fruiting stage, and correlated with this, the rise in protoplasmic viscosity, cold resistance of the plants declined sharply.

The results of our work and the data in the literature lead us to conclude that the primary causes of a high cold resistance in hardened plants are the more flexible adaptive reactions to sharp changes in external environmental conditions present in these plants; these depend on the special characteristics of metabolism as well as the colloidochemical properties of the protoplasm which determine these characteristics.

SUMMARY

Hardening of tomato and corn seeds by application of variable temperature exerts a large effect on the colloidochemical properties of the protoplasm and in particular on its viscosity. During the first stages of ontogenesis the protoplasm viscosity of hardened plants may be higher or lower than that of the control plants. However upon cooling, the hardened plants react more rapidly and to a greater extent than do the control plants, the viscosity of their protoplasm dropping more sharply during a brief cooling period and rising to the critical level at a slower rate under more prolonged action. These changes in the protoplasm viscosity reflect the existence of more perfect adaptive reactions of hardened plants with respect to sharp changes in the environment. It is this property which is responsible for the higher cold resistance of the plants.

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DIFFERENTIATION OF GROWING POINTS IN BIENNIAL ROOTCROPS

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Differentiation of growing points in biennial plants has been very little studied. Our experiments with leaf removal in seedlings grown in the light after vernalization have shown that cabbage produces shoots without leaves, while rootcrops do not produce shoots [1, 2]. This gave us the basis for proposing that in rootcrops (as distinguished from cabbage) the differentiation of growing points begins after vernalization, in the light, in the process of growth at increased temperatures (above 15-20°).

For studying this problem the experiments were carried out under the controlled conditions of the phytotron. Investigations have been carried out since 1955. Seeds and seedlings were received from the Gribovskaya Selective Station. The seedlings of different plant varieties were grown in flower pots, in the absence of vernalizing conditions, at temperatures above 18-20°. Two months after germination, in the phase of five to six leaves, plants were vernalized at 2-3°, with additional illumination for 70-80 days. Plants of the same varieties grown from seed were grown in the field, in July-August, after which they were removed in order to avoid the nightly vernalizing drops of temperature. Then they were placed for vernalization at 2-3° into the dark moist compartments for 60 days. Determination of development stages in seedlings and transplanted plants was made on the basis of biological reactions (bolting, bud formation, flowering) after plants were placed in a warm greenhouse on a long day.

During vernalization of plants, the morphophysiological analysis of plants was carried out.*

For determination of differentiation of the central upper stem buds and stages of morphogenesis, samples of plants were taken every 5 to 10 days from the beginning of vernalization. Preparation of plants was done under a binocular microscope, under magnification of 17 x 2.65 (i.e., 45 x). The prepared growing points were photographed with the microscope camera "MFN-3". Objects were measured with an ocular micrometer.

Observations on the morphogenesis of seedlings in carrot, variety Nantskaya, have shown (Fig. 1, 1) that in the process of vernalization at low temperatures the differentiation of growing points does not take place, and the latter almost do not change in form and size. During this time the growing point reaches 100-150 μ in diameter. Ten to twelve days after plants are placed at warm temperatures (above 20°) with a long day the growing point swells (the first stage of morphogenesis) and is somewhat increased in size (Fig. 1, 2), and 18 to 20 days later it reaches 200-250 μ (second stage of morphogenesis, Fig. 1, 3). During the third phase meristematic buds of the future small umbels of the inflorescence appear on the growing point. This is a qualitative turning point, i.e., a transformation from the vegetative to the reproductive phase of morphogenesis. After 27 to 30 days, the growing point is considerably increased in size, reaching 400 μ (Fig. 1, 4). At this time, flower shoot appear. After 5 to 7 days the apical growing point is transformed into the inflorescence -a large umbel with cylinders along the periphery, the future small umbels (Fig. 1, 5). The whole umbel reaches dimensions up to 1 mm (the fourth stage); the shoot, up to 4-5 cm in height. Such are the first stages of differentiation in vernalized seedlings in the light stage of plant development. If the vernalization is not completed, then at the warm temperature the growing point only swells and is somewhat increased in size, but does not differentiate. On a short day and in the absence of leaves in carrot seedlings the vernalization, growing point differentiation, and shooting are retarded.

In transplanted carrot plants of the same variety, in spite of the presence of a root with a large amount of reserve nutrient substances, the growing point also does not differentiate in the process of vernalization. During growth of vernalized plants in conditions of higher temperatures, the growing point passes the same stages of morphogenesis as seedlings. However, the differentiation takes place twice as fast, altogether in 18 to 20 days (Fig. 2), which is connected with the high role of reserve organs (roots). For example, if in seedlings the second stage of morphogenesis begins after 18-20 days, in transplanted plants it begins from 6 to 7 days after

*L. A. Alpat'eva and N. N. Popova participated in the work.

vernalization, and the third stage is already connected with bolting. Here, in the transplanted plants the three stages of morphogenesis and bolting in the presence of leaves can take place even in the darkness, while in seedlings all this takes place only on a long day, at a temperature not lower 15 to 18°. In the absence of leaves the bolting and differentiation of plants grown from seeds are retarded.

The subsequent stages of morphogenesis connected with the formation of flower buds and flower organs in seedlings and transplanted plants take place only at high temperature (above 20-25°) and good illumination (in intensity and quality); at low temperature and weak illumination the formation of flower buds is retarded, which indicates the presence of the following stages of development (third and fourth) in plants. More than that, the inflorescences appearing in flower buds develop into vegetative organs.

In other umbelliferous plants, differentiation of growing points begins also after the vernalization of plants in the light stage, and approximately at the same time. For example, in transplanted celery (Fig. 3) the stages of morphogenesis take place only several days later as compared with transplanted carrot plants. Differentiation of buds in parsnips and parsley also takes place after vernalization. Other investigators [3-4] came to analogous conclusions concerning the stages of carrot morphogenesis. However, they analyzed differentiation of growing points without relationship to the temperature and light regime and occurrences of various stages of plant development.

What are the peculiarities of morphogenesis in the beet? Above all, one should note that differentiation of growing points and bolting of the beet often begins only after vernalization—as in carrots—and passes through the analogous first phases of morphogenesis.

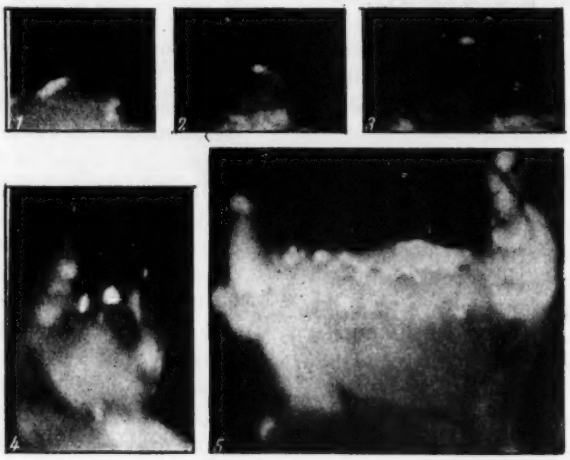

Fig. 1. Differentiation of growing points in vernalized seedlings of carrot, variety Nantskaya (at temperature above 25°). 1) At the end of vernalization, 2) at 11 days after vernalization (the first stage of morphogenesis), 3) at 18 days after vernalization (second stage of morphogenesis), 4) at 27 days after vernalization—bolting (third stage of morphogenesis), 5) at 37 days after vernalization—after three days of bolting (fourth stage of morphogenesis).

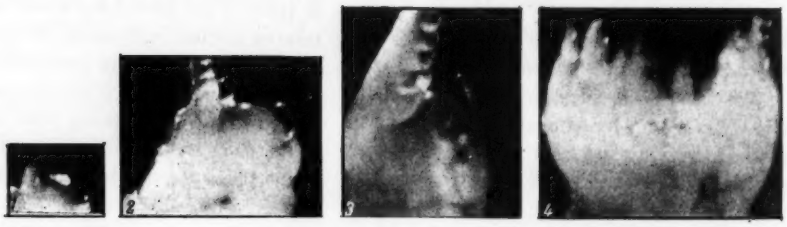

Fig. 2. Differentiation of growing points in vernalized transplanted plants of carrot, variety Nantskaya (at 20°).1) before transplanting (the first stage of morphogenesis), 2) at 6 days after transplanting (second stage of morphogenesis), 3) at 13 days after transplanting—bolting (third stage of morphogenesis), 4) at 22 days after transplanting—4 days after bolting (fourth stage of morphogenesis).

In seedlings of the red table beet Bordeaux, the growing point differentiation takes place faster than in seedlings of carrots. For example, the second phase of morphogenesis in beets takes place already on the ninth to tenth day after vernalization (i.e., twice as rapidly); bolting in the beet also takes place earlier (Fig. 4).

The third stage of morphogenesis in beet seedlings, characterized by the further increase of growing points and its differentiation (appearance of protuberances), takes place in 18 to 19 days, and growing points reach by this time the size of 300 μ; the bolting of plants begins. In 8 to 10 days the fourth stage of morphogenesis takes place, connected with initiation of inflorescences. At this time, the plants have shoots up to 5 cm high, and the apical growing point increases up to a large size (1,000 μ), is conical in form, and is all filled with protuberances—embryos of shoots of the inflorescence or "tassel". These stages take place on long and short days at temperatures above 15°. Below the apical point, the lateral buds are initiated, and begin to differentiate. In the absence of leaves, the vernalization and bolting of seedlings are retarded.

In the transplanted red beet plants, in spite of the presence of the powerful reserve organ (root) the differentiation of growing points takes place at the same time as in seedlings: This emphasizes the important role of the beet leaves, which are no less important to the root-crops and which is distinguished in this connection from the leaves of carrots, cabbage, etc. According to our observations, the leaves of the beet contain twice as

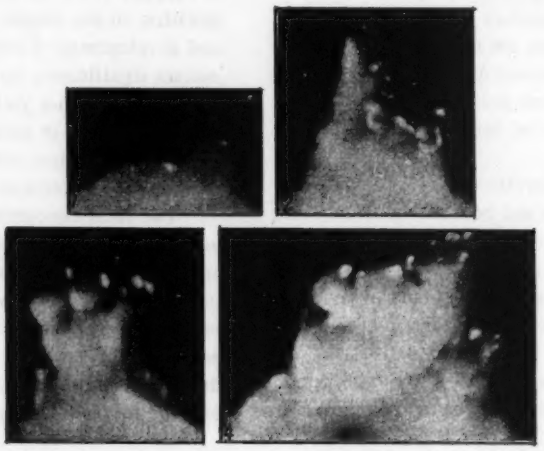

Fig. 3. Differentiation of growing points in vernalized transplanted celery plants during growth (at temperature above 20°). 1) Before transplanting into soil (the first phase of morphogensis), 2) at ten days after transplanting (second phase of morphogenesis), 3) at twenty-one days after transplanting—bolting (the third phase of morphogenesis), 4) at thirty-five days after transplanting—six days after bolting (fourth phase of morphogenesis).

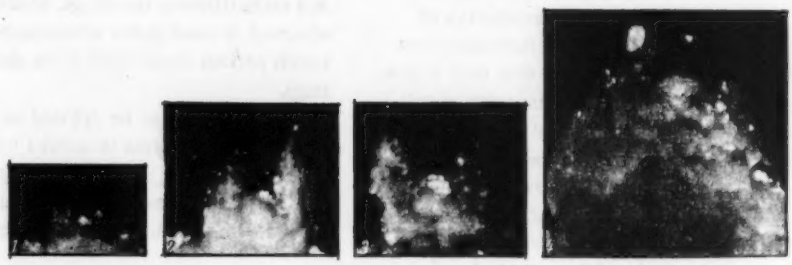

Fig. 4. Differentiation of growing points of vernalized seedlings of the beet variety Bordeaux (at a temperature >20°). 1) At the end of vernalization (the first stage of morphogenesis), 2) at nine days after vernalization (second phase of morphogensis), 3) at 18 days after vernalization—shooting (the third phase of morphogenesis), 4) at 29 days after vernalization—in five days after bolting (fourth phase of morphogenesis).

much sugars as the leaves of carrot and cabbage. In the absence of leaves, the bolting and differentiation of growing points are retarded. Observations of morphogenesis of seedlings and transplanted plants of the sugar beet, medium-ripe variety R-06 have shown that, in spite of a somewhat longer stage of vernalization as compared with variety Bordeaux, the differentiation and bolting takes place in both almost simultaneously. Their types, the size of their growing points, and stages of their morphogenesis are the same.

It is known that vernalized seedlings of table and sugar beet, when leaves are present, bolt on long and on short days. However, for occurrence of the subsequent phases of morphogenesis and formation of flower buds, as well as for flowering in the carrot and beet, a long day and a higher temperature are needed. In the opposite case, the flower buds are not formed, the tips of flower shoots turn into rosettes of leaves, and inflorescences proliferate, which indicate the presence in beet of the subsequent stages of development (third and fourth).

Thus our hypothesis is confirmed that in the transplanted umbelliferous plants and beet the differentiation of growing points takes place after vernalization in the light, during the process of growth and bolting of plants at high temperatures as it takes place in cereals. Other authors [6, 7] had also come to the analogous conclusion.

In connection with facts established by us concerning the times of differentiation of growing points in rootcrops and other biennial plants, the possibility is opened up of directing the process of morphogenesis (to make it more rapid or to retard it) by means of nutrition factors, regulation of the water regime, and application of chemical substances, by which the yield of rootcrops and production of seeds may be influenced.

With this purpose, in 1959 special vegetative experiments were carried out regulating the water regime and mineral nutrition in the first 3 to 4 weeks of growth, when bolting of plants and differentiation of growing points of rootcrops takes place. Rootcrops were planted on April 17. They have shown that with active moisture of 40% of the total moisture capacity of soil, as compared with 60%, differentiation of growing points and bolting of the beet Bordeaux was retarded by 5 days. This had an effect on the subsequent growth and formation of inflorescences; this is related to the productivity of the plant according to the seed yield.

When a double dose of phosphorus (superphosphate) was applied prior to planting, the bolting of the beet plants variety Bordeaux as compared with controls (NPK) was not retarded and took place on the 17th day after transplanting. Application of the phosphorus 15 days after transplanting, when control plants were on the verge of bolting, sharply retarded the differentiation of buds, bolting and growth of experimental plants, in spite of their normal growth and the presence in them of leaves and roots. For example, the bolting of beet in the first case took place May 5, while with the late application of phosphorus, it occurred May 13. Analogous data have been obtained in transplanted carrot plants; without fertilizer, the bolting rook place May 10, while after the double dose of phosphorus prior to planting, it occurred May 4.

Consequently, phosphorus nutrition has a considerable effect on the time of differentiation of growing points. The earlier it is applied, the better it is. Analogous results have been obtained according to the time of application of nitrogen. Previously we have observed a similar phenomenon in cotton [8], and other authors have in cereals. The data obtained concerning the effect of nutrition on the tempo of morphogenesis and on the growth and development of rootcrop plants may have an important significance for the practical side of agriculture in obtaining higher yield of seeds.

The process of morphogenesis is undoubtedly connected with metabolism, which we are studying. The data obtained will be communicated in a special essay.

The facts concerning the stages of morphogenesis established by means of morphological–physiological analysis in biennial rootcrops allow us to use them in selection purposes for selecting of form according to the length of vegetative periods in the early stages of development, and also affecting the morphogenesis by the regime of mineral nutrition and irrigation, bearing in mind increased yield of seeds.

SUMMARY

Differentiation of the growing points of biennial root crops was studied. In umbelliferous rootcrops and the beet plant the transformation of vegetative buds into generative ones occurs at a high temperature after vernalization during the light stage of development and in the presence of leaves, just as in the case of cereals. Four phases of morphogenesis have been established for beet and umbelliferous seedlings. Similar phases have been observed in seed plants of rootcrops but the differentiation periods were found to be shorter than in the seedlings.

The results can be applied to plant breeding work for selection of forms in accord with the length of the vegetation period during early stages of development and also when mineral nutrition and irrigation are employed with the purpose of enhancing the seed yield.

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FORMS OF CALCIUM IN THE SEEDLINGS OF WOODY PLANTS

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The physiological significance of calcium in the life of plants is extremely important and complex. In particular, we showed [1, 2] that the gradient of the concentration of Ca in the roots and stems of larch and spruce seedlings is basipetal and in the leaf organs is acropetal.

Later Abutalybov [3] showed that the active forms of Ca (water soluble and absorbed) are accumulated in the protoplasm of the young, active parts of the plant and the nonactive forms (soluble in acetic and hydrochloric acids) in the cell sap of the old parts (which exhibit decreased activity) of the plants. The necessity for studying the various forms of Ca in the plants for an explanation of several points of the internal cellular processes was stressed by Kostytschew and Berg [4].

The characteristics of the concentration of the various forms of Ca in the organs and tissues of the seedlings of several larch and spruce species are presented in the present work.

The normal Ca, soluble in water, 2N acetic and 2N hydrochloric acids and the so-called "nonsoluble residue" (that fraction of the calcium that is soluble only in strong hydrochloric acid) were measured. Extracts for determination of the forms of Ca were obtained after this from the same batch. Two grams of air-dried plant material was soaked in an Erlenmeyer flask at first with 100 ml of distilled water at room temperature, stirred for a period of 10 minutes, and left overnight. On the following day the solution was filtered through an ash-free filter and the precipitate was washed with 100 ml of distilled water. The filtrate was collected in a meauring flask with a volume of 250 ml, reduced to spots and wet with toluene. Then the precipitate was collected quantitatively with the filter, again wet with 100 ml of 2N CH_3COOH, stirred for a period of 10 minutes, left overnight, further filtered through the same filter, and washed with 100 ml of 2N CH_3COOH; the filtrate was collected in a 250-ml flask. The hydrochloric acid extract was prepared in a similar manner. The precipitate remaining on the filter after all of these operations was dried together with the filter, burned and calcined in a muffle. That form of calcium that we call, somewhat conditionally, "nonsoluble residue" was measured in the ash. Water extracts, opalescent and colored in yellow light of different intensities, were obtained: Needles gave dark, almost brown shades, especially for pine and spruce; the stems gave a lighter color and the roots one still lighter and clear.

The measurement of the Ca in the extracts was carried out without preliminary concentration of the solution, calcination of the precipitate or disintegration of the organic complexes in the solutions, because a similar method did not reflect the accuracy of the measurement of the Ca forms, and this simultaneously accelerated and simplified the path of the analysis.

Two-year-old seedlings of Scotch Pine (Pinus silvestris L.), Siberian larch (Larix sibirica Led.), common spruce (Picea excelsa L.), common ash (Fraxinus excelsior L.), common elm (Ulmus effusa Willd.) and summer oak (Quercus robur L.), grown in the Cherkizovo nursery (near Moscow) on heavy clay medium podzol soils fertilized with pig manure served as material. The method of taking the plant material in the nursery and preparation of the plants for analysis were described in previous works [2, 5]. The needles, stems and roots were divided vertically into several parts. Approximately the top third of the stems and needles of the uppermost part of the plant, and the thinnest and youngest parts for the lowest-layer roots were designated No. 1. The oldest part of the organs, designated No. 3 for pine and larch and No. 5 for ash roots and No. 4 for ash stems, was that adjoining the root collar. The remaining levels occupied an intermediate position based on age and were designated by the corresponding numbers.

RESULTS AND DISCUSSION

The data obtained supported our previous findings [6] on the ability of spruce and elm to use Ca, in comparison to oak, ash, larch and pine (Table 1). It was noted further that the seedlings of the various species are also essentially differentiated by the concentration of calcium forms. Thus, 62% for oak and 48% for spruce consisted of forms soluble in hydrochloric acid, which do not readily take part in metabolism, and only 16% for oak and 20-26% for spruce are of the forms soluble in water and acetic acid. Larch, on the other hand, was differentiated by the highest concentration of the active, water-soluble form of Ca (47.5%), and contained about a third (34%) soluble in acetic acid and only 5% soluble in hydrochloric acid. The main accumulation of Ca soluble in acetic acid (47%) and in water (32%) and the

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relatively small accumulation of Ca soluble in hydrochloric acid (18%) was a characteristic feature of the calcium metabolism of ash. These three fractions were found primarily in equal quantities for elm and pine.

The smallest quantity of Ca (from 3 to 6%) was observed in the form of the so-called "nonsoluble residue" in all species. The exception was larch, which had 13%. Consequently, the tree species are significantly differentiated by their calcium metabolism. Oak and spruce, obviously, belong to the group of species characterized by the rapidly moving and rapidly completed calcium exchange and by the rapid use of calcium in the process of intracellular exchange. Ash and larch, on the other hand, are characterized by slow calcium exchange and a longer period of use of Ca in metabolism. Pine and elm in this respect occupy an intermediate position. Obviously, these species must have significant differences in the carbohydrate, protein and especially in the acidic metabolism, as a result of which the formation of slightly soluble calcium compounds is more or less possible.

However, if the total measurement of any element in the plant, including calcium, makes it possible to understand its part in the metabolism only in the very normal form, then in a similar manner the concentration of different forms in the entire plant gives only a general picture characteristic for each species. Undoubtedly, the determination of the relative concentration of the forms of Ca in the leaves, stems and roots makes possible a deeper understanding of the role of Ca in woody plants. It appeared that the different species do not accumulate Ca similarly in the various organs (Table 2). Elm, ash, spruce and larch had the highest absolute concentration of normal Ca in the leaves; it generally appeared in the stems for oak and in the roots for pine. Second in this respect was the stem for elm and ash, the leaf organs for oak and pine, and the roots for spruce and larch. Thus, the tree species by the structure of their organs use different absolute and relative quantities of Ca.

The coniferous species, in particular, were characterized by a higher concentration of total calcium in the roots than the broadleaf species. The distribution of the different forms of Ca showed that for oak there is a very high concentration in all organs of physiologically nonactive Ca, soluble in 2N hydrochloric acid (leaves—61%, stems—69% and root—56%). The simultaneous concentration of other forms of Ca varied from 13 to 18%, somewhat increased in the roots (24%) and decreased in the stems (19%) (water-soluble fraction). We see again for oak the characteristic tendency to transform Ca rapidly from the active form into forms slightly active in further metabolism. It is interesting that for several barberries in the phase of flowering (Ox. acitosella L. and Ox. stricta L.) crystals of oxalate of calcium in the cells of the deep layers of the parenchyma of the stem and petioles and in the chlorophyll tissue of the leaf blade are not soluble in 20% acetic acid [7].

Obviously for oak, in the process of metabolism large quantities of oxalic acid are also formed, which also holds Ca in a slightly accessible form. This must be noted in view of the ability of oak to accumulate large quantities of Mn [8] and also the presence in the tissues of oak of a large quantity of substances able to accept oxygen. All of this characterizes oak as a plant possessing a highly active oxidizing system. In contrast to oak, spruce had a high concentration of Ca soluble in the hydrochloric acid extract (63%) only in the needles, while the greatest part in the stems and roots consisted of Ca soluble in acetic acid (51 and 55%), about a quarter soluble in water (about 27%) and a small part soluble in hydrochloric acid (14-10%). The needles, stems and roots of the spruce had an extremely low concentration of the so-called "nonsoluble residue". Thus, for spruce, only the needles have a rapid calcium exchange. In the stems and roots, this process is accomplished definitely more slowly and somewhat differently than in the needles and does not proceed as deeply. Obviously, one can also consider that the Ca soluble in acetic acid is a product of some intracellular process and is more available for reutilization than the Ca soluble in hydrochloric acid. In connection with this we must note that the presence of amorphous carbohydrate-callose was observed in the bark parenchyma of healthy plants of Abatilon striatum var. Thompsonii in the layers of crystals of calcium oxalate [9].

The concentration of Ca soluble in hydrochloric acid in the stems of elm reached more than half of the total quantity (53%), about 40% in the roots and only 28% in the leaves. The leaves of elm, in contrast to oak and spruce are characterized by a higher concentration of readily soluble and assimilable Ca (32.4% water soluble and 37% soluble in acetic acid). In the stems these two forms of Ca comprise 42% and in the roots 54%. Thus, elm occupies a position after oak and spruce in respect to the character of the calcium metabolism, having in its tissues a higher concentration of Ca available for taking part in further metabolism. Ash is characterized by a predominant accumulation of forms of Ca even more available for taking part in metabolism than elm, spruce or oak. The total of the water and acetic acid fractions in ash is as high as 80% in the leaves, 76% in the stems and 81% in the roots, while in the latter two cases more than half is in the acetic acid fraction, while the Ca soluble in hydrochloric acid makes up only 15-23% of its total quantity.

Still more sharply expressed is the primary accumulation of physiologically active forms soluble in water and acetic acid in the organs of pine. Here, in contrast to intermixed species, the portion of Ca soluble in water was significantly greater. This increase took place in the main part in proportion to the decrease in

TABLE 1. Forms of Calcium in Entire Seedlings of Woody Species (cut July 28)

Species	Ca in mg per 100 g of absolutely dry substance in the extract					Percent of Ca of total in extract			
	H_2O	CH_3COOH	HCl	non-soluble residue	total	H_2O	CH_3COOH	HCl	non-soluble residue
Common elm	205.7	272.9	301.0	35.4	815.0	25.2	33.5	36.9	4.4
Summer oak	100.3	100.5	383.8	35.5	620.1	16.2	16.2	61.9	5.7
Common ash	204.2	305.3	115.5	18.9	643.9	31.7	47.4	17.9	3.0
Common spruce	163.2	210.5	396.2	52.6	822.5	19.8	25.6	48.2	6.4
Scotch pine	159.9	117.6	140.1	13.9	431.5	37.1	27.3	32.5	3.1
Siberian larch	233.1	168.3	24.0	64.2	489.6	47.5	34.4	4.9	13.2

the portion of calcium soluble in acetic acid and partly in proportion to that soluble inhydrochloric acid. The so-called "nonsoluble residue" in pine was from 3-5% of the total quantity. However, the concentration in pine of Ca soluble in hydrochloric acid was not insignificant—31% in the needles and 23% in the stems. Larch occupied the extreme position and more than 70% of the Ca in the needles was soluble in water and only 1.5% soluble in hydrochloric acid. In the stems and roots, as in most species with the exception of oak, primarily Ca soluble in acetic acid (43-61%) was accumulated; its remaining forms were: 11-14% soluble in water and 5-23% soluble in hydrochloric acid.

It is interesting that in contrast to all of the investigated species, larch contained a very large quantity of "nonsoluble residue", especially in the stems and roots, which contained 20 and 23%. Nevertheless, larch, and to some extent pine, are viewed correctly as species for which the calcium metabolism in all organs, particularly in the needles and roots, proceeds without the formation of large quantities of the poorly soluble compounds. The main part of the Ca in the needles apparently remains in the protoplasm and does not take part in metabolism [10]. It is also possible that the oxalic acid is not formed in the larch to such an extent that it is obviously connected with the features of the carbohydrate-protein metabolism.

We will look at the distribution of the various forms of Ca in the tissues of the root, stem and needles of pine, larch and ash by age (see figure). In the needles of pine and larch, regardless of the date of taking up the plants, the gradient of the concentration of all forms of Ca (in mg per 100 g of absolutely dry substance) is increased in proportion to the age of the tissues, that is, from the uppermost, young needles to the lowest, old needles. The only exception was the so-called "nonsoluble residue", the concentration of which was decreased in the needles of pine in proportion to the age of the needle. Because in the older needles of pine and larch the Ca soluble in hydrochloric acid is accumulated more intensively (in absolute quantity) than in the young needles, the percent concentration of this form of Ca based on its total concentration is increased and the portion of the other forms of calcium is correspondingly decreased (see figure). Similar facts were noted by Emmert [11] in the leaves of apple. The greatest quantity of total and soluble Ca was observed during the vegetation period in leaves formed from nonfruiting buds on the shoots of the preceding year, somewhat less in the old basal leaves of the shoots of the current year and still less in the uppermost young leaves on the shoots of the present year.

In the stems of pine, larch and ash, the distribution of the forms of Ca by age of the parts of the plant appeared to be less certain than in the needles. For pine the Ca soluble in water, partially that soluble in hydrochloric acid, and the "nonsoluble residue" as in the needles has an acropetal gradient of the concentration in the absence of Ca soluble in acetic acid. Correspondingly, the percent concentration of these forms of Ca based on its total concentration in the tissues of various levels of the pine stems is increased or decreased (see figure).

There was a distribution of the forms of Ca by age in the larch stems different from that in the pine stems. The gradients of the concentration of Ca soluble in water and the "nonsoluble residue" were, as in the needles, acropetal while the gradients of the concentration of the physiologically less active and poorly assimilable Ca soluble in acetic acid and also the nonactive and practically nonassimilable Ca soluble in hydrochloric acid were somewhat decreased in the tissues with an increase in their age. In the absence of needles, the greatest part of the Ca in the larch stems is the fraction soluble in acetic acid (43-54%), while in the needles a similar position is occupied by the Ca fraction soluble in water (64-77%). In passing, two interesting facts must be noted: the more intensive

TABLE 2. Forms of Calcium in the Various Organs of Two-Year-Old Seedlings of Tree Species

Portion of plant	Ca in mg per 100 g absolutely dry substance in the extract					Percent of Ca of total in the extract			
	H_2O	CH_3COOH	HCl	non-soluble residue	total	H_2O	CH_3COOH	HCl	non-soluble residue
Common elm									
Leaves	517.0	589.0	448.0	41.0	1595.0	32.4	36.9	28.1	2.6
Stems	103.0	160.0	333.0	34.0	630.0	16.4	25.3	52.9	5.4
Roots	83.0	156.0	175.0	34.0	448.0	18.6	34.9	39.2	7.3
Summer oak									
Leaves	121.0	128.0	446.0	38.0	733.0	16.5	17.5	60.8	5.2
Stems	101.0	208.0	774.0	47.0	1130.0	9.0	18.5	68.5	4.0
Roots	90.0	48.0	210.0	30.0	378.0	23.8	12.7	55.5	8.0
Common ash									
Leaves	566.0	561.0	245.0	45.0	1417.0	40.0	39.6	17.3	3.1
Stems	71.6	250.2	98.2	3.5	423.5	16.9	59.1	23.2	0.8
Roots	70.4	183.6	46.4	13.3	313.7	22.4	58.5	14.8	4.3
Common spruce									
Needles	145.0	208.0	686.0	50.0	1089.0	13.3	19.1	63.0	4.6
Stems	168.0	316.0	89.0	48.0	621.0	27.1	50.9	14.3	7.7
Roots	192.0	394.0	71.0	60.0	717.0	26.8	55.0	9.9	8.3
Scotch pine									
Needles	180.9	80.0	123.8	14.5	399.2	45.3	20.0	31.0	3.7
Stems	94.6	103.1	65.1	13.8	276.6	34.2	37.3	23.5	5.0
Roots	152.1	224.4	53.9	12.3	442.7	34.4	50.7	12.2	2.7
Siberian larch									
Needles*	517.2	142.7	11.1	61.4	732.4	70.6	19.5	1.5	8.4
Stems	26.1	104.2	56.8	57.0	244.1	10.7	42.7	23.3	23.3
Roots	50.3	222.9	19.2	71.2	363.6	13.8	61.3	5.3	19.6

*Oldest needles of the lower level not included in the analysis.

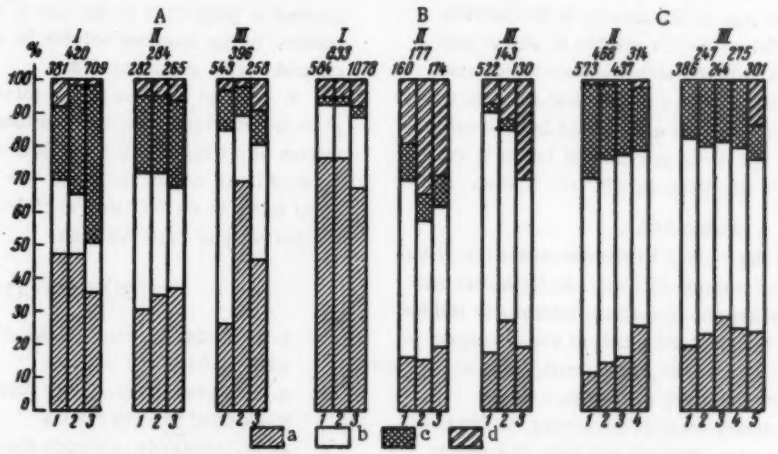

Fig. Forms of calcium (as % of its total quantity) in the various tissues of pine, larch and ash seedlings. A) Pine (collected July 28); B) larch (collected August 28); C) ash (collected July 28); I) Needles; II) stem; III) roots; a) Calcium soluble in water; b) calcium soluble in CH_3 COOH c)calcium soluble in HCl; d) "nonsoluble residue"; 1) Youngest parts; 2) older parts; 3,4,5) oldest parts. The numbers above the columns indicate the total quantity of calcium in 100 g of absolutely dry substance.

accumulation of the so-called "nonsoluble residue" in the old tissues of the stem (19-36%) rather than in the young tissues and, in the tissues of all levels of the stem, the practically equal and small quantities of Ca soluble in water (16-19%) and Ca soluble in hydrochloric acid (8-11%). All of this stresses the characteristics of the calcium metabolism in larch.

The concentration of Ca soluble in water in the stems of ash was increased in an acropetal direction but the concentration of other forms of Ca increased in an opposite direction. A very high percentage concentration of Ca soluble in acetic acid was characteristic for the ash stems, as for the larch. In the absence of pine, and especially larch, ash had in all organs a very low concentration of the so-called "nonsoluble residue" (see figure). In the roots of pine, larch and ash, the gradients of the concentration of Ca of all forms was expressed in a basipetal direction with the exception of the "nonsoluble residue", the concentration of which increased with the age of the roots. The Ca content (in percent of total) was changed correspondingly.

Thus, one can conclude that in the leaf organs of pine and larch, the portions of Ca active and able to take part in metabolism (water fraction) is noticeably increased in proportion to the age of the tissues, regardless of the absolute increase, but the portions of the less available and nonactive Ca soluble in acetic and hydrochloric acids is increased. In the stems and roots of pine, larch and ash on the other hand, the portions of the water-soluble Ca, which is easily assimilable for taking part in metabolism (just as the so-called "nonsoluble residue" of Ca is nonassimilable for taking part in metabolism), increases in proportion to the age of the tissues; in connection with this, the portion of the Ca soluble in acetic and hydrochloric acids is noticeably decreased. However, it must be noted that the Ca fraction that we have called the "nonsoluble residue" was established in noticeable quantities only in the stems and roots of larch; it was in extremely small quantities in ash (see figure).

SUMMARY

1. We traced the change in the concentration of the various forms of Ca: soluble in water, in 2N acetic and hydrochloric acids, and the so-called "nonsoluble residue" (soluble in strong hydrochloric acid) in various organs of two-year-old seedlings of oak, elm, larch, pine and spruce grown near Moscow in heavy clay soils.

2. The most active users of Ca among the broadleaf species are elm, then ash and oak, and among the coniferous species, spruce, followed by larch and pine.

3. Oak and spruce are characterized by a rapidly proceeding calcium exchange. Ash and larch, on the other hand, are characterized by slow calcium metabolism and by a longer time of use of calcium in metabolism.

4. The greatest quantity of Ca absorbed in elm, ash, spruce and larch is accumulated in the leaves, in oak in the stems, and in pine in the roots. Coniferous species are characterized by a higher concentration of Ca in the roots than broadleaf species.

5. The distribution of the various forms of Ca in the separate organs of the seedlings is different. The highest concentration of Ca soluble in HCl is for oak, leaves 61%, stems 69%, roots 56%; for elm, in the stems 53%; for spruce, in the needles 63%; soluble in acetic acid, for ash in the stems and roots 59% and for larch in the stems 43 % and in the roots 61%; soluble in water, for ash in the leaves 40%, for pine in the needles 45%, and for larch in the needles-71%.

6. The gradient of the concentration of all forms of Ca in the needles of pine and larch is acropetal; its greatest quantity is accumulated in the needles of the lower levels. In the stems of these species and for ash, the gradient of the concentration of Ca soluble in water is acropetal and for that soluble in acetic and hydrochloric acids is basipetal. The gradient of the concentration for all forms of Ca in the roots of these species is in a basipetal direction.

7. The portion of Ca active and readily available for taking part in metabolism (soluble in water) is decreased in comparison with the other forms in proportion to the age of the tissues of the leaf organs of pine and larch regardless of the absolute quantity of Ca of this form, but the portion of the less active and less available soluble in acetic acid, and also that soluble in hydrochloric acid, increases. The portion of the water-soluble fraction in the stems and roots of pine, larch and ash increased in proportion to the age of the tissues and the portions of the fractions soluble in acetic and hydrochloride acids are decreased.

8. With an increase in the total age of the whole plant of pine seedlings, the absolute and relative concentration of Ca soluble in water is decreased, but it is increased in all organs for larch. The concentration of other forms of Ca for larch decreased, but increased in the majority of cases for pine.

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THE FORMATION AND QUANTITATIVE CHANGE OF CYANOGENIC GLYCOSIDES IN SPROUTING AND MATURING FLAX

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There is a cyanogenic β-glycoside, linamarin ($C_{10}H_{17}O_6N$), soluble in water and bitter to the taste, in the vegetative parts and seeds of flax. It is separated into prussic acid, acetone and glucose under the action of dilute acids and the enzyme linamarase. The concentration of prussic acid in all parts of the flax depends on the conditions of sprouting, the variety characteristics and the age of the plants [1].

In order to study the effect of growing conditions on the content of prussic acid in flax seeds Peterson and Spenser [2] planted flax in containers, and, after flowering for a month, created various soil moisture conditions for the plants and subjected them to the effect of low temperatures and other variables. There was 6 mg/100 g more prussic acid in the seeds grown under low soil moisture conditions than in those grown with high soil moisture. Sprouting and mechanical injury contributed to the increase (up to 4 mg/100 g) in prussic acid in seeds. In the case where the temperature dropped down to 13° at the time of ripening, the quantity of prussic acid in the seeds doubled [2].

The formation of prussic acid is connected with the metabolism of nitrogenous substances. The probable path of formation of prussic acid (nitrile group) takes place through decarboxylation of amino acids and oxidation of the amines that are formed, with the oxygen given off at the time of the decomposition of hydrogen peroxide with the enzyme catalase.

Our investigations established that there are more glycosides in the seed of various varieties of flax grown under the steppe conditions of the south on chernozem soils than in those varieties grown under the conditions of the moist climate close to the ocean near Leningrad (Table 1).

There were more glycosides in the flax seed in dry years (during the period of ripening) under the conditions of the Kuban Experimental Station than in the years that were wetter during the period of ripening. Seeds of long-fibered flax, as a rule, contain more glycosides than seeds of the oil varieties of flax. This corresponds directly to the concentration of nitrogenous substances in them.

The difference between varieties with respect to concentration of prussic acid, under the conditions of the USSR, ranges from one and one-half times to twice (see Table 1). Nitrogen fertilizer increases the concentration of prussic acid. According to our data, prussic acid is partially evolved into the air on sprouting. One can judge this by the bitter smell of the shoots when the seeds are germinated in closed containers. In our experiments, picrate papers placed in closed containers above five- to seven-day-old shoots gave a clear qualitative reaction to HCN.

It is possible that the cyanogenic glycosides, and especially the prussic acid formed from them, play an important protective biological role, protecting shoots from damage by insects and diseases.

Different varieties of flax grown under conditions of the northern and southern zones were used to study the process of accumulation of glycosides on germination. Separate batches of these seeds were germinated at 22-23° during the day in light and at 12-13° at night. The germination of the shoots was stopped during the phase with green cotyledon leaves after seven and 12 days. The height of the shoots was 5-10 cm on the seventh day and 14-16 cm on the twelfth day. The analysis showed that the quantity of prussic acid on the seventh day in the shoots increased seven to 11 times and on the twelfth day still continued to increase for several varieties. Because oxidizing processes take place with the conversion of oils and the separation of protein with the formation of amino acids during germination, the quantitative growth of the cyanogenic glycoside is connected with the oxidation of the amides that are formed according to the formula:

$$CH_2NH_2COOH \rightarrow CO_2 + CH_3NH_2$$

$$CH_3NH_2 + 2O \rightarrow HCN + 2H_2O.$$

Active oxidizing processes and vigorous respiration, which is accompanied by a strong increase in the activity of the enzyme catalase, take place with the growth of the shoots.

TABLE 1. Concentration of Prussic Acid* and Glycosides in the Seeds of Different Varieties of Flax (in mg/100 g of dry substance; average for 1950 and 1951)

Origin and name of variety	Kuban		Pushkin	
	HCN	Linamarin	HCN	Linamarin
Svetoch k-5333	46	420	23	210
Holland k-3920	43	392	22	200
Pskov k-725	37	330	23	210
VIR-1650	26	237	19	174
Afghan k-1268	34	310	21	192
Azerbaijan k-2026	37	330	20	182
Georgia k-1374	36	329	23	210
Sicily k-2524	26	237	22	200

*Measured by the mercurometer method [4].

TABLE 2. Formation of Glycosides on Sprouting of Seeds of Pushkin and Kuban Reproduction (in mg HCN per 100 g of original dry seeds taken for germination. 1952 experiment)

Origin, name and catalog number	Kuban, 1951 harvest			Pushkin, 1951 harvest		
	Seeds prior to germination	7-day sprouts	12-day sprouts	Seeds prior to germination	7-day sprouts	12-day sprouts
Svetoch k-5333	38	350	462	22	—	350
Holland k-3920	39	287	287	21	225	287
Pskov k-725	31	287	350	21	350	350
VIR-1650	22	225	287	18	225	287
Afghan k-1268	32	225	225	18	225	287
Georgia k-1374	32	287	350	20	287	287
Sicily k-2545	25	187	225	22	187	225
Azerbaijan k-2026	35	225	225	18	225	287

The concentration of linamarin for the seven-to 12-day-old sprouts reaches 3-4.5% (of the dry substances) and this takes place partially in proportion to the loss of dry substances, which make up 15-25% of the weight of the seeds. Apparently, in no case was linamarin given off first from the shoots.

The results of the analysis show (Table 2) that the seeds of the same forms grown in two different zones (Kuban and Pushkin, near Leningrad) gave shoots that were not differentiated by the concentration of linamarin (in the dry substances of the shoots) when grown under laboratory conditions, in spite of significant differences in the concentration of linamarin in the seeds. However, the shoots of different varieties of flax were differentiated by the concentration of linamarin, which varied by a factor of two. The concentration of prussic acid is decreased in proportion to the growth of the flax plants, but its concentration remains high in the young leaves, especially in years with cool and damp weather. For example, the average air temperature during the period of flax flowering was about 14° in the conditions of Pushkin (near Leningrad) in 1952. Average samples of young leaves taken from the upper third of the stem of plants at the time of flowering contained in the dry substance from 150 to 370 mg of prussic acid, and from 1.4 to 3.3% when converted to linamarin (Table 3). Here it must be noted that the concentration of glycosides in the upper leaves for long-fibered plants (Svetoch, Holland, Pskov) was significantly higher than for the medium-fibered and short-fibered plants. The concentration of glycosides in the leaves, as in the seeds, is changed in relation to meteorological conditions.

We must note that the collection of leaves for long-fibered plants and the four from Sicily was carried out at the end of flowering, and for the medium-fibered plants at the beginning of flowering.

The study of the dynamics of prussic acid in the leaves and in the developing pods* was carried out in

*Seeds in dry pods make up 40% during green maturity and 62-65% during complete maturity.

TABLE 3. Concentration of Linamarin in the Leaves of Different Forms at the Beginning of Flowering (August 12, 1952, Pushkin)

Origin, name and catalog number	Dry substance of the leaves, %	HCN, mg/100 g	Linamarin (HCN x 9.14) as % of dry substance
Pskov k-725	18.7	285	2.60
Svetoch k-5333	18.7	285	2.60
Holland k-3920	17.1	370	3.30
VIR-1	15.1	217	1.98
VIR-1650	15.0	217	1.98
Afghanistan k-1268	16.1	217	1.98
Georgia k-1374	15.8	150	1.37
Sicily k-2524	15.9	150	1.37

TABLE 4. Change in Concentration of Dry Substances (%) and Cyanogenic Glycosides (in mg HCN/100 g) in the Leaves* and Pods of Flax (1957 experiment, Pushkin)

Name of variety and phase of development	Dry substance		HCN to green weight		HCN to dry weight	
	in leaves	in pods with seeds	in leaves	in pods with seeds	in leaves	in pods with seeds
Smolensk L - 1220, long fibered:						
Flowering	20.5	18.0**	12.4	49.8**	51	277**
Milk ripeness	19.8	21.5	8.6	44.3	45	206
Green ripeness	20.5	40.7	4.1	10.8	20	27
Late yellow ripeness	26.0	56.1	8.6	14.4	33	26
VIR-1650:						
Flowering	16.0	18.5**	11.6	51.1**	73	279**
Milk ripeness	17.7	22.8	7.6	22.2	43	97
Green ripeness	16.6	38.5	5.4	11.9	32	31
Late yellow ripeness	20.6	39.5	7.0	9.7	34	25
Large-seed 3:						
Flowering	16.0	19.8**	12.6	50.0**	78	252**
Milk ripeness	17.4	26.1	7.3	22.7	42	87
Green ripeness	16.0	37.1	4.3	8.1	27	22
Late yellow ripeness	23.3	42.0	4.9	7.5	21	18

*For comparison of the results, leaves for analysis were collected from the upper half of the stem, because the leaves had already dropped from the lower part of the stem during the yellow-ripeness phase.

**Buds and flowers.

1957 on three different varieties (Table 4). When comparing the data for variety VIR-1650, one must note that the quantity of HCN in the leaves in 1957 was only one-third (73 mg) (Table 4) of that in 1952, i.e., 217mg (Table 3).

From the data of [2], a sharply increased quantity of HCN in the leaves in 1952 is explained by the cool weather during the vegetation period.

For flax varieties an increase in the dry substances of 2.6 times (from the time of milk-green to the late yellow ripeness phase) takes place in proportion to the growth and ripening of the pods, but the concentration of HCN is decreased 2-3.5 times. The highest proportion of HCN (glycosides) to the green and dry substance is in the flowers and buds (from 252 to 279 mg/100 g of HCN or from 2.3 to 2.6% glycosides to dry substance). The concentration of glycosides in the leaves decreases from flowering up to ripening.

Variety differences based on the concentration of linamarin in the leaves and pods are completely expressed during the late yellow-ripeness phase. The concentration of prussic acid in the flax stems also decreases in proportion to growth and development. In the usable part of the straw of long-fibered flax L-1120 in 1957 there were the following quantities of HCN in mg/100 g of dry substance: 4.5 mg during the green-ripeness phase, 3.0 in the yellow, and 2.5 in complete ripeness.

The fact that the highest concentration of prussic acid for all varieties is in the young growing organs (in the embryos, leaves, buds and shoots) must be stressed.

On the basis of measurements of the weight of the aerial mass of 100 plants and the portions in leaves, stems and pods with seeds during the period from the beginning of flowering to complete ripeness, there was found to be a change in the total quantity of prussic acid. Table 5 shows that in the aerial mass of three different varieties,

TABLE 5. Change in the Total Quantity of Prussic Acid in the Aerial Mass and in the Separate Plant Organs (in mg per 100 fresh plants)

Variety name and phase of development	Leaves	Stems	Pods with seeds	Entire plant
Smolensk L-1120				
Flowering	4.1	4.0	11.4*	19.5
Green ripeness	1.2	5.3	6.8	13.3
Yellow ripeness	1.9	4.1	7.6	13.6
VIR-1650:				
Flowering	7.1	7.1	15.3*	29.5
Green ripeness	3.9	6.1	17.7	27.7
Yellow ripeness	2.8	3.7	14.0	20.5
Complete ripeness	3.3	3.9	9.7	16.9
Large-seed 3:				
Flowering	5.8	10.2	15.0*	31.0
Green ripeness	3.3	8.5	15.8	27.4
Yellow ripeness	1.8	5.4	7.7	14.9
Complete ripeness	1.9	4.8	6.9	13.6

*Buds and flowers.

the total quantity of prussic acid decreases 1.5 to 2 times, more than half of all the prussic acid is in the pods with seeds (in all phases of ripeness) and in the buds and shoots during the period of flowering.

The maximum quantity of prussic acid in milligrams per 100 plants was found during the period of flowering and then its quantity decreased in all parts of the plant. One can assume that, as during the time of germination, part of the glycoside is broken down and prussic acid is given off into the surrounding medium.

The results of our investigations, and data in the literature suggest that the increased concentration of glycoside is in a direct relationship with the increased concentration of nitrogenous substances, chlorophyll, and ascorbic acid, and also with the increased activity of the enzyme catalase and respiration.

The young leaves and plants, unripe seeds, and fruits contain more glycosides than the old vegetable organs, ripe seeds, and fruits.

SUMMARY

The concentration of cyanogenic glycoside in flax seeds depends on the growing conditions and variety characteristics.

The yield of seeds of flax of the same varieties grown under different meteorological conditions are differentiated by the quantity of prussic acid.

A strong increase in the concentration of linamarin in proportion to the new growth takes place in the germinated seeds. Shoots seven to 12 days old are very toxic.

The highest concentrations of linamarin are in the flowers and buds; the highest concentrations of glycoside in the leaves occur during the period of flowering.

The flax seeds contain noticeable quantities of glycoside during the period of complete ripeness of the seeds.

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DYNAMICS OF LIGNIN AND CELLULOSE DURING THE PROCESS OF GROWTH OF CORN*

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The main wall of the cell layers is made of cellulose. With increasing age of the plants, this is incrusted with lignin, one of the complex substances, which gives rigidity to the wall. It has been noted that the quantity of cellulose and lignin does not remain constant during the process of plant growth [1-4]. Part of the cellulose and probably the lignin of the cell wall is converted into soluble compounds, and the latter are again used by the plant as plastic material.

Cases of delignification of the cell walls and mobilization of part of the cellulose in the stems are well known, for example, for plants of rye [2] and corn from experiments in 1956 and 1957 under the conditions of Krasnoyarsk [5].

In 1958 we carried out a study of the effect of lignification and delignification of the cell walls in corn stems in the process of growth. In constrast to the first two years, we took three varieties of corn in 1958: early-ripening Minusinka, average-ripening VIR-42 and late-ripening Krasnodarskaya 1/49. The method of establishing the experiment, maintenance and observations of the plants, dates of taking samples for analysis, etc., were all similar to our 1957 experiment [5].

EXPERIMENTAL RESULTS

In 1958 corn was planted on May 23, with a soil temperature of 12.7° at a depth of 10 cm and air temperature of 28°. However, the air and soil temperature fell soon after planting; the Spring was cold and remained so for a long time. The average temperature in June was 15.4°. The average monthly rainfall decreased from 19 mm in May to 15 mm in June. All of this had a severe effect on the corn shoots and sharply retarded their growth.

The shoots of different varieties of corn were not the same. Minusinka appeared completely on June 13, VIR-42 on June 17 and Krasnodarskaya 1/49 on June 19-20.

There were more favorable climatic conditions in July. The average air temperature was 19.7°, the month's precipitation increased to 53 mm, the relative humidity of the air increased to 63%, and the number of clear days increased significantly (Table 1). All of this had a favorable effect on the growth of corn, which recovered quickly. As soon as the formation of the male flowers of the late-ripening variety (Krasnodarskaya 1/49) began, we began to take regular samples simultaneously from the three varieties for measuring the lignin, cellulose and water-soluble substances.

The dynamics of the growth of the corn plant that were analyzed were 60 cm for Minusinka for the stem height up to the tassel, 22.2 cm for VIR-42 and 15.0 cm for Krasnodarskaya 1/49; the size of the male flower was 26.3 cm for Minusinka, 10.5 cm for VIR-42 and 3.1 cm for Krasnodarskaya 1/49. The female flower was formed only in the Minusinka early-ripening variety and, without its sheath, it measured 1.8 cm. The vegetative mass increased rapidly for the late-ripening variety (Table 2).

A gradual accumulation of vegetative mass took place with further growth of the plants and by the beginning of September plants of the Minusinka early-ripening variety reached maximum dimensions for all indicators, while the average-ripening variety and even more the late-ripening variety continued to grow and build up vegetative mass (see Table 2).

The buildup of lignin, cellulose and water-soluble substances proceeded along with the growth of the vegetative mass of the plants.

1. Changes in the Accumulation of Cellulose

The cell walls of the mechanical, conducting and, partially, the basic tissues of the stem thickened with increased age of the plants. The degree of thickening of the walls was different and depended on the function of the cells and the conditions of their growth. The accumulation of cellulose in the plants is connected with the thickening and nonthickening of the cell walls, and, besides this, in the form of hemicellulose, it is deposited in the cells in reserve.

The accumulation of cellulose as a whole in the stems of corn in the second half of the vegetative period proceeds irregularly, but with the same regularity for all three varieties. For example, on August 2 the percent

*Second communication. For the first communication on this question, experimental method and analysis, see [5].

TABLE 1. Data of Meteorological Observations of the Krasnoyarsk Experimental Field, 1958

Climatic conditions	Month				
	May	June	July	August	September
Soil temperature at depth of 10 cm, °C	—	15.4	20.5	18.0	9.2
Average air temperature, °C	8.7	15.3	19.7	16.1	7.7
Average relative humidity, %	50	60	63	68	72
Total precipitation, mm	19	15	53	66	38
Number of cloudy days	7	14	6	6	13
Number of clear days	2	3	4	11	1

of cellulose reached 21.7% in Minusinka, 18.3% in VIR-42 and 15.1% in Krasnodarskaya 1/49 (Table 3).

The difference in the accumulation of cellulose by varieties is explained by the different growth by phase and stage of organogenesis. Particularly, the early-ripening variety was in the phase of panicle formation, the average-ripening variety in the phase of tube formation the late-ripening variety in the phase of male flower formation.

The accumulation of cellulose in all varieties continued and on August 13 it reached 25.3% in Minusinka, 23.8% in VIR-42 and 17.5% in Krasnodarskaya 1/49.

Increases in cellulose are not observed for the Minusinka early-ripening variety during the period of flowering, fruit formation and grain formation, but cellulose is decreased 23.06% during the phase of milk ripeness and wax ripeness. The accumulation of cellulose for VIR-42 average-ripening variety proceeds up to August 25 and for Krasnodarskaya 1/49 late-ripening up to September 5, that is, in proportion to the approach of the indicated species' male flower formation.

An accumulation of cellulose, reaching 29.5%, again takes place prior to ripening of the early-ripening variety and prior to grain formation for the average-ripening variety. We were not able to trace any further change in the cellulose because of the death of the plants from autumn frosts.

2. Changes in the Accumulation of Lignin

Lignin, which impregnates the thickened cell walls, is closely connected to the accumulation of cellulose. The quantity of lignin in stems is changed with increased age of the plant. Probably, it is able to be partially converted into soluble forms and is used in nutrition of the cells of the forming reproductive organs, spores and grain.

Based on the data of our analyses, the quantity of lignin in the corn stems by variety was as follows on August 2: 42.1% for Minusinka,† 30.1% for VIR-42 and 27.3% for Krasnodarskaya 1/49. The quantity of the lignin is increased with further growth of the plants and reached the greatest accumulation on August 13. For example, it was 47.3% for the early-ripening variety, 37.3% for the average-ripening and 33.4% for the late-ripening. The difference in the accumulation of lignin by variety depends, as in the case of cellulose, on the phase of growth and stage of organogenesis (see Table 3). During the period of flowering, fruiting and formation of grain for the Minusinka early-ripening variety from August 13 to August 25, the accumulation of lignin is sharply decreased, reaching 28.3%, while the quantity of water-soluble substances in the vegetative organs and female flowers is increased: from 5.45 to 8% in the stems, from 6.3 to 8.3% in the leaves and from 8.5 to 9.0% in the cobs. A decrease in the water-soluble substances in the male flowers is connected with the gradual dying of the latter (Table 4).

Consequently we must assume that the accumulated lignin, partially transformed into the soluble form, is used as a reserve product in the process of flowering and in seed formation.

Such a rule in the accumulation and consumption of lignin is also observed in the other two varieties of corn (see Table 3). For example, the accumulation of lignin for VIR-2 average-ripening variety continued up to August 25 (panicle formation phase) and reached 43.26%. This accumulation continued to September 5 (also to the panicle formation phase) for the late-ripening variety, and was 41.2%. Then the accumulation of lignin was shaply decreased and reached 24.3% for Krasnodarskaya 1/49, because the process of flowering,

† The high percent of lignin that showed up in all varieties is partially a result of the method of measuring the action of the highly concentrated hydrochloric acid (suggested by Wilschtetter and Zechmeister). With such a separation of lignin some contamination of the latter with secondary products of the hydrolysis of carbohydrates and proteins is possible. We did not check the purity of the lignin precipitate.

TABLE 2. Dynamics of the Growth of Analyzed Corn Plants During the Second Half of the Vegetative Period (1958)

Date of taking sample for analysis	Phases of growth and stages of organogenesis	Length, cm					Number of leaves	Size of leaf blade without sheath, cm	
		Stem up to tassel	Flowers: Male	Flowers: Female: with sheath	Flowers: Female: without sheath	Entire plant		length	width
Minusinka early-ripening									
2.VIII	Panicle formation	60	26.3	4.9	1.8	104.0	7.2	55.2	6.1
13.VIII	Dropping of filament; flowering of panicles	83.6	33.1	18.8	8.86	120.9	7.0	61.2	7.7
25.VIII	Beginning of milkripeness	69.6	33.0	30.0	14.7	124.5	6.8	65.4	7.74
5.IX	Early wax ripeness	88.5	36.5	31.5	17.4	129.0	7.2	62.0	8.3
18.IX	Wax ripeness	62.9	29.0	44 0	14.8	120.0	7.2	67.7	8.2
VIR-42 average-ripening									
2.VIII	Tube formation	22.2	10.5	—	—	120.0	9.0	77.4	8 .6
13.VIII	Pollen formation	41.6	22.4	17.6	1.0	127.4	11.4	77.6	11.2
25.VIII	Panicle formation	87.3	28.16	19.6	6.0	142.3	11.6	73.0	9.4
5.IX	Dropping of filaments and panicle flowering	97.4	36.3	39.3	12.8	148.0	12.0	74.3	10.0
18.IX	Grain formation	87.0	36.6	29.0	9.8	138.0	11.5	67.3	9.7
Krasnodarskaya 1/49 late-ripening									
2.VIII	Male flower formation	15.0	3.1	—	—	109.0	9.3	77.6	6.4
13.VIII	Tube formation	22.0	11.3	—	—	113,6	10.3	78,3	7.5
25.VIII	Pollen formation	55,6	35,8	14,3	2.5	130.0	12.0	77,3	9,0
5.IX	Panicle formation	75.0	34,3	18.5	5,5	138.7	13.5	79.0	9.75
18.IX	Dropping of filaments and panicle flowering	79.6	33.0	25.7	8.9	142.5	13.5	71.0	8.9

TABLE 3. Dynamics of Lignin and Cellulose in Corn Stems During the Second Half of the Vegetative Period

Variety	Date of taking sample for analysis	Phases of growth and stages of organogenesis	As percent of dry weight: cellulose	As percent of dry weight: lignin	Lignification scale based on Boyarkin
Minusinka	2.VIII	Panicle formation	21.7	42.1	5
VIR-42	2.VIII	Tube formation	18.3	30.1	3
Krasnodarskaya 1/49	2.VIII	Formation of male flowers	15.1	27.3	2
Minusinka	13.VIII	Dropping of filaments and panicle flowering	25.2	47.3	5
VIR-42	13.VIII	Pollen formation	23,8	37.3	4
Krasnodarskaya 1/49	13.VIII	Tube formation	17.5	33.4	3
Minusinka	25.VIII	Beginning of milk ripeness	25.2	28.3	3
VIR-42	25.VIII	Panicle formation	29.8	43.26	5
Krasnodarskaya 1/49	25.VIII	Pollen formation	22.2	37.8	4
Minusinka	5.IX	Early wax ripeness	23.06	37.53	4
VIR-42	5.IX	Dropping of filaments and panicle flowering	27.9	24.3	3
Krasnodarskaya 1/49	5.IX	Panicle formation	26.06	41.2	5
Minusinka	18.IX	Wax ripeness	29.5	33.4	3
VIR-42	18.IX	Grain formation	29.5	31.2	3
Krasnodarskaya 1/49	18.IX	Dropping of filaments and panicle flowering	26.4	29.7	3

TABLE 4. Dynamics of Water-Soluble Substances (%) in Various Organs of Corn During the Second Half of the Vegetative Period

Date of taking sample for analysis	Phases of growth and stages of organogenesis	Stem	Leaf	Panicle	Cobs
	Minusinka early-ripening variety				
2.VIII	Panicle formation	4.29	10.51	15.56	6.19
13.VIII	Dropping of filaments and panicle flowering	5.45	6.3	13.6	8.5
25.VIII	Beginning of milk ripeness	8.0	8.3	5.3	9.0
5.IX	Early wax ripeness	8.7	9.7	2.9	8.8
18.IX	Wax ripeness	7.2	12.9	2.2	8.3
	VIR-42 average-ripening variety				
2.VIII	Tube formation	5.0	5.39	6.89	—
13.VIII	Pollen formation	5.54	6.3	8.9	6.3
15.VIII	Panicle formation	5.4	6.7	9.0	6.0
5.IX	Dropping of filament and panicle flowering	7.7	6.3	12.9	7.3
18.IX	Grain formation	6.3	7.7	13.5	8.0
	Krasnodarskaya 1/49 late-ripening variety				
2.VIII	Formation of male flowers	4.19	9.26	—	—
13.VIII	Tube formation	5.4	6.0	0.7	—
25.VIII	Pollen formation	5.7	7.6	10.3	6.3
5.IX	Panicle formation	7.7	6.3	7.7	7.0
18.IX	Dropping of filament and panicle flowering	8.0	12.2	—	7.3

fruiting and seed formation took place in this period for the indicated varieties under the conditions of Krasnoyarsk in 1958. While the accumulation of water-soluble substances during this period was increased in the stems and reproductive organs and reached 12.9% for VIR-42 in the panicles, 7.3% in the cobs, 7.7% in the stems, for Krasnodarskaya 1/49 the quantity of water-soluble substances reached 12.2% in the leaves, 7.3% in the cobs, 8% in the stems; the panicles were dead at this time. The quantity of water-soluble substances in the grains of Minusinka dropped in the period of wax ripeness. This is connected with the conversion of water soluble substances into a nonsoluble form (Table 4).

An accumulation of lignin up to 37.5% again took place before ripening of the grain of the Minusinka early-ripening variety, and the concentration fell again to 33.4% in the phase of wax ripeness of the grain (see Table 3). The lignin was also increased to 31.2% for medium ripening variety VIR-42 prior to formation of the grain. We were not able to note this for the late-ripening variety because the plants had already died from the autumn frosts at the time of grain formation.

The degree of lignification of the stems in the process of the corn growth was checked by the Boyarkin method [6]. For this, anatomical sections of the stem were made at the level of the first node and treated with phloroglucine with hydrochloric acid. Based on the phase of growth, the sections showed the same changes in the lignification of the stems as the data of the chemical analysis showed (see Table 3).

SUMMARY

In the process of vegetation, changes take place in the accumulation of lignin, cellulose and water-soluble substances. At the beginning of vegetation, as a rule, an accumulation of the high-molecular carbohydrates takes place, from which the mechanical strength of the corn stem is increased and reserve products are accumulated for further use in the period of flowering, fruiting and seed formation. A process of partial delignification of the stem takes place with the beginning of the phase of milk ripeness for the Minusinka early-ripening variety and with the phase of dropping of filaments and panicle flowering for the VIR-42 average-ripening variety and Krasnodarskaya 1/42 late-ripening variety. The quantity of lignin is noticeably decreased and the quantity of water-soluble substances is increased. The increase in the quantity of water-soluble substances, in all probability, is connected with the conversion of cellulose and lignin into soluble compounds and the use of the latter as reserve products in the process of flowering and seed formation.

1. Lignification and partial delignification of the corn stem occurs periodically during the process of vegetation of the plant.

2. Both the works of A. M. Paleev and the data of our experiments, carried out over a period of three years, show that the cell wall, in addition to having a mechanical function, partially plays a role of reserve products. It can hydrolyze, and the products of hydrolysis,

in all probability, take place in metabolism with the formation of new organs and cells of the plants.

3. The concentration of cellulose and lignin in the cell walls of corn stems is changed with the age of the plant. At the beginning of growth a rapid accumulation of cellulose and lignin takes place. After this the quantity of lignin and cellulose decreases up to the time of the formation of the reproductive organs. We suggest that here partial delignification of the cell walls takes place and the products of the disintegration migrate from the stem to the place of their consumption.

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THE CULTURE OF CHLORELLA PYRENOIDOSA IN INTERMITTENT LIGHT

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The duration of the photochemical and enzymatic reactions of photosynthesis was first measured by Emerson and Arnold [1, 2] in a study of photosynthesis in intermittent light, in which the light and dark periods were of various lengths. It was shown that, on the basis of CO_2 assimilated or O_2 released per unit light energy, light is utilized most efficiently when it is given in flashes of 0.00001 second alternating with dark intervals of about 0.1 second. These experiments were repeated and extended by a number of workers [3-6] with various interpretations of the data obtained. It is in general established, however, that photosynthesis involves both photochemical and dark reactions.

In studies of the induction period of photosynthesis and of photoperiodism [7-9] it was shown that both photosynthetic rate and growth (of higher plants and algae) are minimal when the light and dark periods are of the order of 1 minute; as the periods are shortened or lengthened, these increase. In the experiments on culture of plants in intermittent light, however, the frequency and duration of the light flashes were not decreased to the level which would correspond to the duration of the light and dark reactions of photosynthesis.

Our study on this point with Chlorella yielded a great deal of information testifying to the complex reaction of the alga to intermittent illumination. Its reaction altered with time, indicating that adaptation was occurring.

Chlorella pyrenoidosa (Pringh) served as experimental material.

The light source was a 200- or 500-joule xenon-filled pulsed fluorescent tube, type IFP-200 or -500[10]. The light temperature of these lamps is 6000-7000°, and the distribution of spectral energies is similar to that of sunlight. The tubes, which were connected to a power supply, functioned at a frequency of 10 cps with a flash duration of the order of 25 microseconds*.

The alga was grown in special glass containers on 250 ml of medium (Fig. 1) in a constant stream of air enriched with carbon dioxide. The medium employed was that used by Tamiya [11] for the mass culture of algae. The number of cells after inoculation varied from 0.5 to 1.5 $\times$ 10^6 per cm^3 of medium. Growth of the culture was evaluated by counting in a Thom chamber or by turbidimetric measurements. In all experiments the temperature was 23-24°. Growth in intermittent light was compared with that in continuous light from luminescent lamps, the light intensity at the surface of the culture vessels being 6-7 thousand lux.

Inasmuch as the intensity of intermittent light optimal for Chlorella pyrenoidosa growth was unknown, an experiment was set up in which culture vessels were placed at various distances from the light source in accordance with the following scheme:

No. of culture vessel	Distance from light source (cm)
4,5,6,7	50
8	35
9	20.5
10	10
11	8
12	3

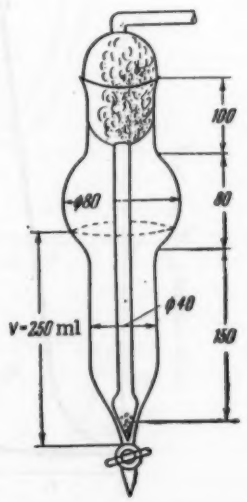

Fig. 1. Culture vessel

*The pulsed lamps and power supply were kindly presented by I. S. Marshak and set up under his direction by V. Orlov, to whom we express our sincere gratitude.

From the results presented in Fig. 2, it is strikingly evident that in every case development of the alga in intermittent light is very weak. The pattern of increase of cell number has two characteristic periods. The first, which lasts 9-11 days after inoculation, is a period of depression and slow increase; the second marks the beginning of relatively rapid growth. Despite the general similarity of the curves, the cultures grown closest to the light source are markedly different (see curve 12).

Cultures grown in intermittent light from large inocula (130 x 10^6/cm^3) behave in a similar fashion (Fig. 3).

During the first period of insignificant growth in intermittent light, however, the culture remains fully viable. This is shown by the fact that when a culture which had been in intermittent light four days was transferred to continuous light its growth was rapid, as was that of cultures grown in continuous light from the beginning (Fig. 4).

It is difficult to ascribe the behavior of Chlorella in intermittent light to a light deficiency, since the curve for increase of cell number with time was not linear but showed a sharp break which, to all appearances, characterized a qualitative change in the condition of the culture. This compelled the assumption that there is an adaptation by Chlorella cells to intermittent light which requires about eight to ten days (period I), after which the adapted culture begins to grow (period II).

The assumption that adaptation occurs seemed reasonable inasmuch as in exposing the culture to intermittent light we are placing it under conditions in which it is necessary that enzymatic activity, biochemical reactions, and possibly even processes as complex as cell development and division be strictly synchronized. Obviously this does not occur instantaneously but requires some time.

A number of experiments were performed to test this hypothesis. It was assumed that if Chlorella is actually adapted to intermittent light, then with repeated transfer the depression of growth associated with the adaptation period should be decreased. Rapid growth should be observed, if not from the first day after inoculation, then after shorter lag periods, and the curve showing increase in cell number in intermittent light should resemble that for cultures grown in continuous light.

Culture conditions were the same as in the preliminary experiment, with the exception that all culture vessels were placed at the same distance from the light source, 6.5 cm. As development of the culture progressed, microscopic observations were made to cell number counts.

As the results presented in Fig. 5 show, with the first inoculation the behavior of Chorella in intermittent light is the same as that noted previously. During the first 30 hours after inoculation, there is even a certain decrease in cell number. During the first nine or ten days there is almost no development of the culture, which is in a sluggish condition, and only on the tenth day does growth begin. Careful microscopic observations made during this time showed (Fig. 5) that the first few days after inoculation the

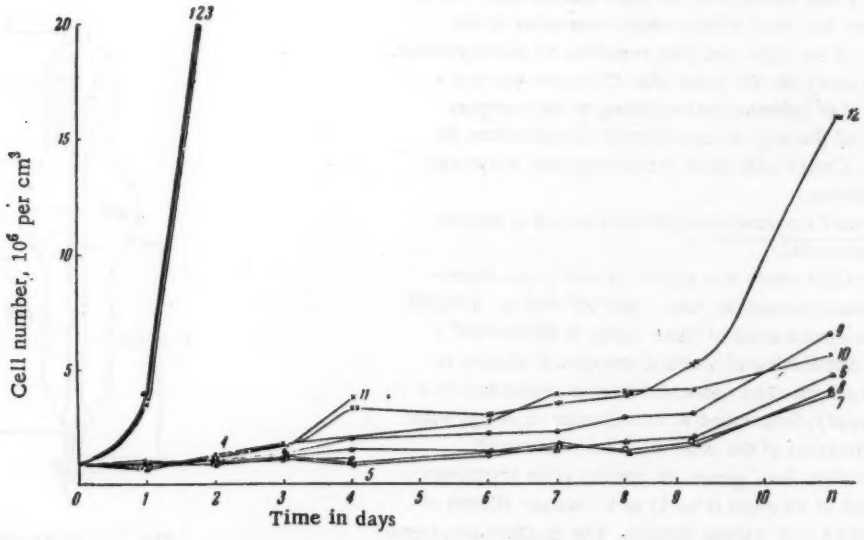

Fig. 2. Growth of Chlorella pyrenoidosa in intermittent light. 1-3—growth in continuous light from pulsed lamps; 4-12—growth in intermittent light as related to distance from the light source.

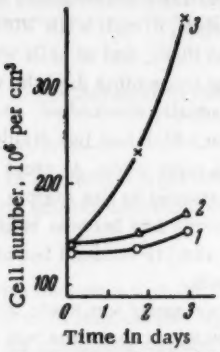

Fig. 3. Growth of Chlorella pyrenoidosa in intermittent light from large inocula. 1, 2—growth in intermittent light, 3—in continuous light.

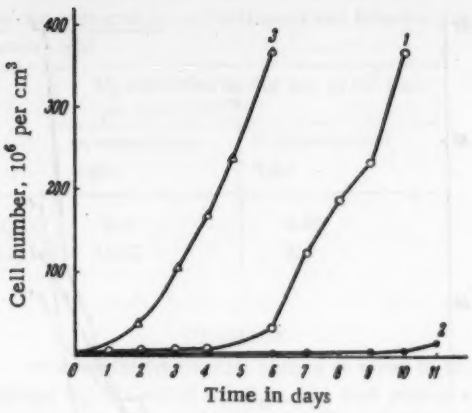

Fig. 4. Growth of a Chlorella pyrenoidosa culture 1—first in intermittent light, then in continuous light; 2—in intermittent light; 3—in continuous light.

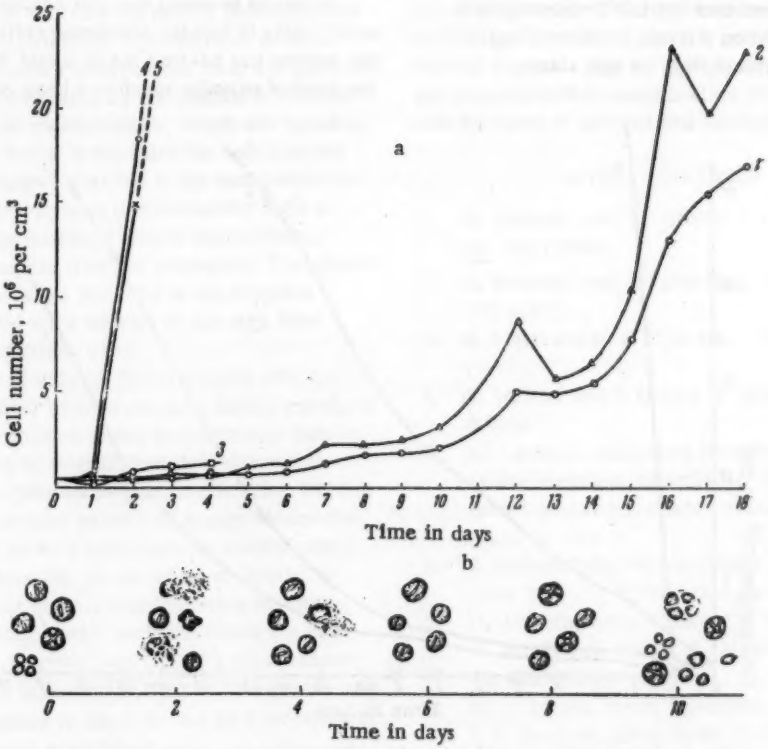

Fig. 5. Growth of a Chlorella pyrenoidosa culture unadapted to intermittent light. 1-3—in intermittent light; 4, 5—in continuous light.

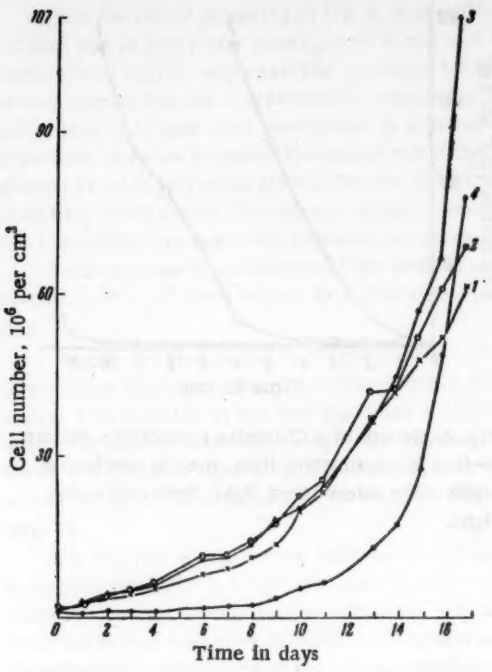

Fig. 6. Growth of a Chlorella pyrenoidosa culture adapted to intermittent light. 1-3—adaptation in intermittent light on a liquid medium; 4—adaptation in intermittent light on agar slants.

culture in intermittent light contained large numbers of disintegrated and partially disintegrated cells; it was full of cellular debris, of cell walls with extruded protoplasm clinging to them, and of cells with diffuse boundaries. During the succeeding days the number of disintegrated cells gradually diminished, and cells which were dividing or which had just divided were encountered more and more often. At about the time that the curve was beginning to rise sharply, practically all the cells in the culture had become bright green, optically dense, with sharply outlined boundaries and clearly visible pyrenoids.

On the 18th day a transfer was made from a culture such as this, which at that time was growing vigorously in intermittent light. The kinetics of growth in the second passage are presented in Fig. 6. As seen from these curves and from a comparison of them with growth curves of the first passage (Fig. 7), the culture began to develop much more rapidly, reaching on the 17th day 100×10^6 cells per cm^3, as compared with 25×10^6 cells per cm^3, without the cell disintegration and growth lag typical of the unadapted culture. It therefore appears to us from these results that there really is an adaptation to intermittent light by Chlorella pyrenoidosa.

It should be noted that this adaptation proceeds more easily in rapidly developing cultures. If, however, the culture was adapted not in liquid medium but in the form of colonies which had been growing on mineral

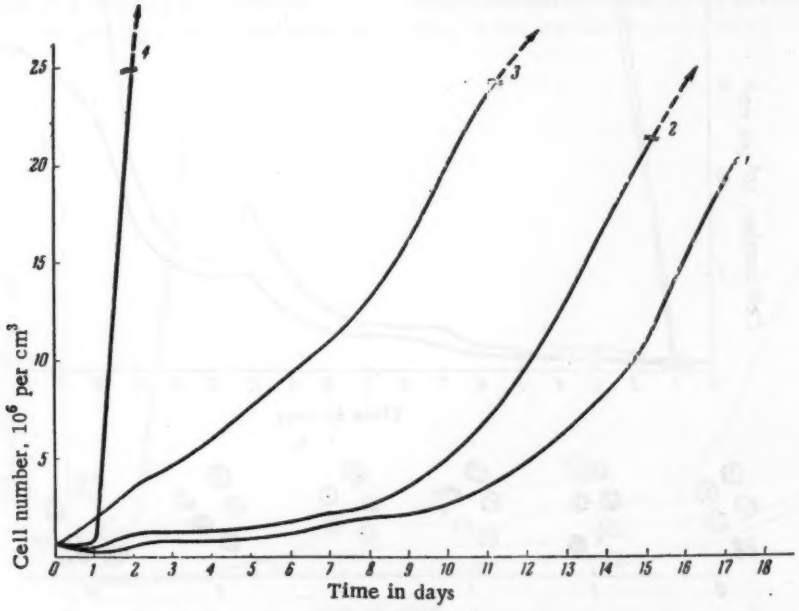

Fig. 7. Comparative growth of various Chlorella pyrenoidosa cultures in intermittent light. 1—unadapted; 2—adapted in the form of colonies on agar slants (cell density on 17th day, 75×10^6 per cm^3); 3—adapted in liquid medium (cell density on 17th day, 100×10^6 per cm^3); 4—in continuous light.

Rate of Photosynthesis, in Terms of O_2 Evolution, of Chlorella pyrenoidosa in Continuous and Intermittent Light as Related to Adaptation of the Culture to Intermittent Light

Character of culture	O_2 evolution in mg per 1×10^6 cells per hour $\times 10^{-6}$	
	in continuous light	in intermittent light
Grown in continuous light (not adapted to intermittent light)	9.3	2.25
Grown in intermittent light (adapted to intermittent light)	12.65	2.3

agar, then with subsequent transfer to liquid medium the kinetics of growth in intermittent light was almost the sames as that for unadapted cultures (Fig. 6).

It was still not clear whether the original growth lag in intermittent light was a result of a suppression of photosynthesis, or whether other processes, cell division processes in particular, were adversely affected.

In view of the studies of a number of workers which showed that photosynthesis in continuous and intermittent light (light flash 0.00001 second, dark period 0.1 second) of identical intensities proceeds at the same rate, it was to be expected that adaptation to intermittent light here involves growth and division processes. To test this hypothesis, a number of experiments were set up to measure the rate of photosynthesis in continuous and intermittent light for both adapted and unadapted cultures. Photosynthetic rate was measured in terms of oxygen evolution by the method of Winkler.

Results of theses measurements, which are presented in the table, show that it is the same for both adapted and unadapted cultures.† This led to the conclusion that the growth lag upon exposure to intermittent light is indeed due to a suppression of growth and division processes, which require time for adaptation. The photosynthetic apparatus is not involved in the adaptive changes which occur upon transfer of the alga from continuous to intermittent light.

A comparison of data on photosynthetic rate and on the maximum number of cells attained during a definite period of time for cultures grown in continuous light as against those grown in intermittent light shows that continuous light is considerably more effective. Whether this is due to the smaller amount of energy obtained in intermittent light, or to a less favorable spectral composition of light from the pulsed tubes, or finally, to some specific effect on cell multiplication by intermittent light as such is still unclear. Under the conditions employed, however, the fact of a clear adaptation of the algae to intermittent light of a frequency and duration corresponding to the light and dark reactions of photosynthesis is well established.

We wish to extend our sincere thanks to Professor A. A. Nichiporovich, at whose initiative and under whose direction this study was carried out.

SUMMARY

The possibility of mass culture of algae in intermittent light, in which the light and dark periods correspond to those of the photochemical and dark periods of photosynthesis was studied. Thus an attempt was made to use for practical purposes well-known facts regarding the mechanism of photosynthesis. The light sources were special pulsed lamps which flashed on 10 times per second, producing a flash of 25 μsec duration.

It is shown that Chlorella pyrenoidosa can grow in intermittent light of 25 μsec duration and 0.1 sec dark intervals. After 17 days the cell density was of the order of 100×10^6 cells per cm^3.

However, growth of the alga can take place in intermittent light only if the cells are adapted during a certain period of time. Growth processes and developmental processes, but not the photosynthetic apparatus, are adapted in this case, since the photosynthetic rate was the same in adapted and unadapted cells.

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†The difference in photosynthetic rate is due to the fact that unfortunately we were unable to equalize the light sources with respect to energy.

THE EFFECT OF ORGANIC SUBSTANCES ON PHOTOSYNTHESIS AND RESPIRATION OF ALGAE OF THE BLACK SEA

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The massive development of many marine algae of the genera Ulva, Enteromorpha, Bangia, Ceramium, etc., in places rich in organic materials has been widely reported [1-5].

In the opinion of algologists specializing in the Black Sea [1, 4], a large number of littoral marine macrophytes require dissolved organic substances in some abundance, and this is evidently the reason why these algae grow luxuriantly in waters polluted by their proximity to cities.

The presence of organic compounds in sea water cannot fail to be reflected in the metabolism of the algae, primarily in photosynthesis and respiration—the most important physiological processes.

In order to elucidate the effect of such materials on respiration and photosynthesis, we made a study of the oxidative metabolism of two algae of the Black Sea, Enteromorpha compressa (L.) Grev. and Phyllophora nervosa (D. C.) Grev., in the presence of certain organic compounds.

In our selection of experimental subjects we were guided primarily by their relation to organic material under natural conditions. According to published information [2, 5, 6] Enteromorpha is often encountered in places rich in organic material and is even a biological indicator of the degree of pollution of sea water. In contrast to Enteromorpha, Phyllophora inhabits the pure waters of the open sea [4, 7].

In view of the fact that the chemical composition of organic substances dissolved in sea water has not been sufficiently investigated [8-11], it was necessary to limit our choices to simple, easily incorporated substances —glucose, sucrose, acetate, and glycine.

Solutions of organic substances were prepared in sea water which had been filtered through paper. Sea water without added organic materials served as a control.

The design of the experiments was as follows. Freshly collected and carefully sorted algae were kept for a definite period (6-12 days) in a crystallizing basin containing sea water and a solution of the organic compound being tested. Solutions were changed every other day. Experiments were run in natural diffuse light and in total darkness.

At the end of each experiment the algae were washed thoroughly with the same solutions and placed in special liter vessels for determination of rates of photosynthesis and respiration according to Winkler's method. Determinations were made in the presence of the organic compounds, freshly prepared solutions being routinely used for this purpose.

Preliminary experiments to determine the effect of organic substances on photosynthesis and respiration of marine algae were performed on Phyllophora with 0.5% and 0.1% sucrose.

The results (Table 1) indicate that sucrose at the concentrations tested enhances the respiration of Phyllophora in the dark. The effect of organic materials on Enteromorpha respiration varies with concentration.

The data of Table 2 show that three % glucose, a mixture of 3% glucose and 0.02% glycine, and 0.5% glucose depress the respiration of Enteromorpha in the dark. Introduction of small quantities of glucose (0.2%) into sea water has the opposite effect, respiration being enhanced.

A considerable increase in oxygen absorption by Enteromorpha in the dark is also observed in experiments with 0.2% sucrose (Table 3).

Our results correspond to published data on the effect of different concentrations of carbohydrates on the respiration of higher plants and unicellular algae [12-18].

The effect of acetate on Enteromorpha respiration varies with concentration (Table 3). While 1% acetate depresses oxygen absorption in the dark, 0.5% acetate almost doubles it. Our data are in agreement with those obtained by other workers [17, 19, 20], who studied Chlorella.

A 0.02% solution of glycine has a comparatively small enhancing effect on Enteromorpha respiration. Results of experiments on the effect of organic compounds on photosynthesis in Enteromorpha and Phyllophora are more uniform. The data presented in Tables 1, 2, and 3 show that in contrast to 0.02% glycine solution, glucose sucrose and acetate at all concentrations tested bring about a decrease in oxygen evolved in the light. These

TABLE 1. The Effect of Sucrose on the Oxidative Metabolism of Phyllophlora

Treatment	Date, 1958	Dry wt, %	O_2 absorbed, in mg, in one hour by 100 g dry matter	Date, 1958	Dry wt, %	O_2 evolved, in mg, in one hour by 100 g dry matter
	Dark			Light		
0.5% sucrose	Apr. 12	28.4	12.20	May 24	31.7	4.66
Control	Apr. 12	27.1	7.10	May 24	31.6	5.32
0.1% sucrose	Apr. 25	28.9	13.60	May 6	32.2	6.97
Control	Apr. 25	28.6	9.40	May 6	32.5	7.22

TABLE 2. The Effect of Various Concentrations of Glucose on the Oxidative Metabolism of Enteromorpha

Treatment	Date, 1958	Dry wt., %	O_2 absorbed, in mg, in one hour by 100 g dry matter	Date, 1958	Dry wt, %	O_2 evolved, in mg, in one hour by 100 g dry matter
	Dark			Light		
3% glucose	July 23	20.7	285.9	July 12	19.4	162.6
3% glucose + 0.02% glycine	July 23	20.2	123.0	July 12	20.5	186.6
Control	July 23	18.8	666.3	July 12	16.7	491.5
0.5% glucose	June 27	17.9	327.5	June 17	16.2	55.2
Control	June 27	17.5	734.0	June 17	18.9	218.9
0.2% glucose	Sept. 12	20.8	81.0	Sept. 16	20.4	1160.2
Control	Sept. 12	20.1	45.0	Sept. 16	19.9	1436.1

results are in agreement with those of other workers [18, 20], who investigated Chlorella.

The extent to which oxygen evolution in the light is depressed by the organic compounds tested depends on concentration. 3% glucose, a mixture of 3% glucose and 0.02% glycine, and 0.5% glucose reduce the rate of photosynthesis in Enteromorpha considerably. Low concentrations of organic materials—0.2% glucose, 0.5, 0.2, 0.1% sucrose, and 0.5% acetate—depress photosynthesis in Enteromorpha and Phyllophora only slightly, and 0.02% glycine even causes a certain enhancement of photosynthesis in Enteromorpha.

The fact that a given concentration of an organic compound depresses the oxidative metabolism to a considerable extent in both the dark and the light deserves special attention. A significant inhibition of photosynthesis and respiration by organic componds indicates that they are toxic to the algae. Apparently this is related primarily to an adverse effect of relatively high concentrations on the colloidal system and the osmotic properties of plant cells.

The marked stimulation of respiration in the dark may serve as a criterion of a favorable effect of small concentrations of organic compounds on the metabolism of the plants investigated. The small reduction in photosynthetic rate in the presence of small amounts of organic materials could be the result of a heightened absorption of these materials in the light [21].

Taking into account the published information [9, 11, 22] on the total amount of organic material in coastal and open waters of the Black Sea, it may be supposed that the presence of such material in sea water brings about an increase in the metabolic activity of algae under natural conditions.

SUMMARY

Results of investigation of the effect of glucose, sucrose, acetate and glycine solutions on photosynthesis and respiration of Enteromorpha and Phyllophora nervosa are presented. Small concentrations of the organic substances (0.2% glucose, 0.5% acetate, 0.5%, 0.2% and 0.1% sucrose and 0.02% glycine) stimulate dark respiration of the algae. High concentrations of organic compounds (3% glucose, 3% glucose and 0.02% glycine mixture, 0.5% glucose, and 1% acetate) inhibit this process.

TABLE 3. The Effect of Acetate, Sucrose, and Glycine on the Oxidative Metabolism (in the Light) of Enteromorpha

Treatment	Date, 1958	Dry wt, %	O_2 absorbed, in mg, in one hour by 100 g dry matter	Date, 1958	Dry wt, %	O_2 evolved, in mg, in one hour by 100 g dry matter
	Dark			Light		
1% acetate	Aug. 14	23.0	340.8	Aug. 19	22.8	658.1
Control	Aug. 14	20.0	416.2	Aug. 19	21.5	760.4
0.5% acetate	Oct. 28	18.2	136.0	Oct. 23	20.5	1533.0
Control	Oct. 28	19.6	77.6	Oct. 23	21.5	2010.0
0.2% sucrose	Oct. 7	16.3	236.9	Oct. 2	17.7	1614.4
Control	Oct. 7	18.8	60.3	Oct. 2	21.9	1739.7
0.02% glycine	Aug. 29	19.2	134.5	Sept. 2	20.0	1753.7
Control	Aug. 29	17.9	101.1	Sept. 2	18.7	1445.0

As a rule photosynthesis is inhibited in the presence of the indicated concentrations of these substances. The only exception is glycine (0.02%), in the presence of which a small increase of the photosynthetic rate is observed.

High concentrations of organic substances considerably lower the photosynthetic rate of algae. Small amounts of organic substances have an insignificant effect on this process.

In conclusion, I wish to express my sincere gratitude to Professor S. I. Lebedev, Director UASKhN.

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A HISTORICAL ACCOUNT OF THE STUDY OF THE ORIGIN OF OXYGEN EVOLVED IN PHOTOSYNTHESIS

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One of the major achievements of recent years in the study of photosynthesis has been the conclusive demonstration of the nature of the oxygen evolved in this process. This is a question which has arisen repeatedly in the years of study of photosynthesis. The first investigators—of the 1870's and 1880's—Priestley [1], Ingen-Housz [2], and Senebier [3], who had pointed out the significance of such factors as the green color of the leaf, the presence of sunlight, and the carbon dioxide of the air, believed carbon dioxide to be the source of the oxygen evolved.

As early as 1796, however, the Russian botanist N. M. Maksimovich-Ambodik in his textbook [4], for the first time in the history of botany set aside a chapter, "Physical or physiological" in the section on plant nutrition, where he confidently asserts that " the primary food of plants is water and air" (p. 173). Water, which is taken up through the roots with salts, is necessary to the life of the plant. The organs in which this nutrient is assimilated under the influence of the sun he believed to be the leaves. In this connection he made the very interesting suggestion that the green color of the leaf is the result of the action of light. The plant dies if the leaves are removed or if the flow of water into them is interrupted. This was the first suggestion that water is one of the main factors in photosynthesis, participating with carbon dioxide in the creation of organic matter under the influence of light. Eight years later Saussure [5] experimentally confirmed this hypothesis, having noted that the role of water amounts to "a transfer of its elements" to the substances being assimilated by the plant. After this the scheme for photosynthesis assumed a form which is basically that currently accepted: The plant, under the influence of sunlight, synthesizes organic substances from water and carbon dioxide of the air.

Being a careful experimenter, and unwilling to accept on faith a single unproven hypothesis, Saussure could not neglect to resolve experimentally a doubt which had arisen in his mind, namely that perhaps water, and not carbon dioxide, was the source of the oxygen evolved. To do this he placed a plant amply supplied with water in an atmosphere free of carbon dioxide. According to his reasoning, if water is decomposed, then even in the absence of carbon dioxide oxygen should be released into the atmosphere. Since he did not observe this, he concluded that photosynthesis involves an interaction of a molecule of water with carbon produced by the reduction of carbon dioxide, the oxygen of the latter being released into the atmosphere. From that time, this assumption was widely accepted, and it was firmly incorporated into scientific thought for more than a hundred years. During this time, however, scientists returned to the problem again and again.

Saussure's conclusions as to the origin of oxygen evolved were first questioned by Professor Gize of Khar'kov University [6], who had been working on the isolation of chemical compounds from plant material and determination of their properties. In his huge five-volume textbook on chemistry, which was considered a model of its time, he touches on the problem of the interaction between the plant and the surrounding air under the influence of light. After thoroughly analyzing the studies of Priestley, Lavoisier, Scheele, and Ingen-Housz, he ventured the very interesting thought that "the oxygen evolved in this case (during photosynthesis—E. S.) . . . comes from water, being removed by the green parts of the plant under the influence of sunlight" ([6], p. 323).

This was the first suggestion that the oxygen evolved in photosynthesis might originate from water. It was later repeated almost word for word by the French botanist Rishar [7].

We find an interesting suggestion concerning the conversion of water during photosynthesis in the work of Professor Dvigubskii of Moscow University [8]. Considering that the leaves are organs of nutrition, just as are the roots, he ventured the opinion that the main component of the nutrient sap of plants is water, which "in the presence of light is decomposed into its component parts—hydrogen and oxygen" ([8], p. 37). He regarded this as related to absorption from the atmosphere of a carbon source and to greening of the plant in the light.

The 1840's were significant in the study of photosynthesis. The investigations of the well-known chemists Liebig and Boussingault attracted attention of the scientific community to this problem. Although their studies were not devoted specifically to the chemistry of photosynthesis, they touched on this subject. Liebig [9] made especially strong efforts to demonstrate that oxygen comes not from carbon dioxide but from the more easily decom-

posed water, the hydrogen of which should then be combined with carbon dioxide to yield a basic organic compound. He believed that synthesis of organic material did not follow immediately upon reduction of CO_2, but that intermediate compounds—plant acids—were involved. Oxalic acid is formed first, then converted to tartaric acid by removal of oxygen, and malic, citric, and other acids are then synthesized, with the final formation of carbohydrates.

Boussingault also advanced the hypothesis that water is the source of oxygen evolved in photosynthesis [10]. An acute observer and experimenter, he noticed that not only starch but also a number of other compounds are formed in photosynthesis, and as a result there is somewhat more oxygen evolved than carbon dioxide absorbed, even though this is not observed by simple gas analysis. Having set up several experiments in which different plants were grown from seed or from seedlings with a preliminary determination of the elementary composition of their organic material, he grew them in soil containing no organic matter and then once more determined the content of carbon, hydrogen, oxygen, and nitrogen. Results of the analysis showed that during the experiment the quantity of hydrogen and oxygen increased as follows (in g):

		H_2	O_2	Addition H_2
From seed	clover	0.176	1.226	0.023
	peas	0.215	1.237	0.060
	wheat	0.078	0.608	0.002
Transplanted clover		0.097	0.444	0.042

Boussingault reasoned that if water were not oxidized, then hydrogen and oxygen should have combined with carbon in the same ratio in which they occur in water. But the data show a disproportionate increase in the amount of hydrogen, and the excess can only be ascribed to the oxidation of water. Boussingault ruled out the possible objection that it could have arisen from ammonia, on the basis of a calculation which showed that even if all the nitrogen in the plant were in the form of ammonia, there would still be a small excess of hydrogen.

This experiment enable Boussingault to conclude that it is necessary to postulate the decomposition during photosynthesis not only of CO_2, but also, in certain cases, of H_2O. He mistakenly assumed, however, that the oxygen released by the plant in this process contains an admixture of carbon monoxide (albeit in insignificant amounts), which is formed by the decomposition of carbon dioxide. This interacts with the hydrogen of water to give a hydrate of carbon, and the oxygen evolved therefore is a product of the decomposition of both carbon dioxide and water.

During the same period Khodnev [11], the author of the first textbook on biochemistry in Russian, pointed out still more strongly the role of water as the source of oxygen. In a study of various substances synthesized by plants, he was impressed by the fact that only the formation of sugar and starch can be explained in terms of the interaction of carbon and water. Such substances as chlorophyll, wax, oil, resin, pectic substances, etc. contain hydrogen in greater amount than corresponds to its ratio to oxygen in water, and indeed some of them consist solely of carbon and hydrogen. From this he concluded that " in every case hydrogen must have come from water, and therefore oxygen should have been released. From this you see that water serves, among other things, as a source of atmospheric oxygen, which is depleted by respiration and combustion" ([11], p. 62).

Shmidt, another Russian chemist, came to the same conclusion [12]. In explaining the experiments of Priestley, Senebier, and especially Saussure, he asserted that "it is impossible to state with certainty that oxygen released by the plant is from carbon dioxide. Perhaps the component parts of carbon dioxide are combined with the hydrogen of water, and the oxygen is therefore derived from the water" ([12], chapter 2, p. 237). The very same view is expressed later in his work "Chemical and Physiological Bases of Agriculture and Cattle Breeding" (1867), a special section of which is devoted to plant nutrition and the processes involved.

The French chemist Berthelot [13] in 1864 proposed a scheme of the decomposition of carbon dioxide and water in photosynthesis, according to which the former dissociates to form carbon monoxide and oxygen and the latter dissociates to form hydrogen and oxygen, with the subsequent formation of carbohydrates:

$$\begin{array}{l} CO_2 \rightarrow CO + O \\ H_2O \rightarrow H_2 + O \\ \hline CO_2 \rightarrow H_2O \rightarrow CH_2O + O_2 \end{array}$$

In spite of the vagueness of this theory, it strikingly showed the requirement for carbon dioxide and water in the first stage of photosynthesis, and also the source of the oxygen formed.

The special communication in the 1866 issue of the journal "Naturalist", entitled "The Source of Oxygen Released by the Plant", testifies to the interest of the Russian scientific community in this problem. The paper acquaints the reader with the studies of the German chemist Erlenmeyer who, on the basis of his own observations and those of Shmidt, Kolbe, Schultz and others, comes to the conclusion that "oxygen released by plants does not necessarily have to come from carbon dioxide, as assumed previously, but may arise from the decomposition of water; the hydrogen released from water acts on carbon dioxide in conjunction with mineral elements, water, and ammonia to form plant materials" (p. 390).

We find a quotation of Erlenmeyer's conclusions of 1863-1866, "concerning the formation of organic plant substances as a result of decomposition of H_2O and not CO_2," written in the margins of K. A. Timiryazev's manuscript,

"The Incorporation of Carbon by Plants and Its Dependence on the Longest Wave Lengths of the Solar Spectrum" in the summer of 1867 (source– the K. A. Timiryazev Museum). Already in this, his first study of photosynthesis, which has remained unpublished, and subsequently in his master's thesis published in 1871, Timiryazev [14], in analyzing the mechanism of photosynthesis and the participation of chlorophyll, emphasizes the significant role of water. Entertaining the views of Berthelot and Boussingault, he was inclined to believe that in this process water is decomposed first of all, but since this assumption was not based on sufficient experimental data he did not develop it further. In subsequent studies he referred to it again and again,however, believing it to be completely valid.

Despite the fact that the hypothesis of the origin of oxygen from water was advanced by such well-known chemists as Liebig, Boussingault, Erlenmeyer, Berthelot, and others, the majority of scientists in the second half of the nineteenth century rejected it. One of the best known agrochemists of the time, Maier, expressed the general scientific opinion, that "we relegate this view to the category of unnecessary and therefore harmful hypotheses which have always, unfortunately, plagued physiological sciences" ([15], chapter 1, p. 68).

Nevertheless,this tendency became still more pronounced toward the end of the nineteenth and the beginning of the twentieth century. The studies of the Russian biochemists A. N. Bakh and V. I. Palladin during this period have especial merit. Bakh [16] regarded the assimilation of carbon by the plant not as a result of splitting of oxygen from a carbon monoxide molecule, but as a coupled redox process involving the hydrogen and hydroxyl of water. On the strength of this, the formation in photosynthesis of reduced products should be necessarily coupled to the formation of peroxides, particularly hydrogen peroxide. A. N. Bakh assumed that it is peroxides which are the compounds which are subsequently decomposed to yield oxygen. From this the conclusion followed that oxygen released during photosynthesis should come from water.

In this connection the view of Palladin [17] on the activation of hydrogen of respiratory substrates (carbohydrated, etc.) and of oxygen in respiration, the antithesis of photosynthesis, is of considerable interest. The studies of Thunberg [18] and Van Niel [19] on the redox processes of photosynthesis later promoted the development of this idea.

In analyzing the chemistry of photosynthesis, Bredig [20] and Hofmann and Schumpelt [21] paid special attention to the conversion of water. They advanced the hypothesis that the decomposition of water to oxygen and hydrogen is the primary light reaction resulting in the evolution of oxygen to the atmosphere. The secondary process, in their opinion, must be the reduction of carbon dioxide by hydrogen.

Not until the beginning of the 1940's was it finally possible to test these hypotheses directly.

The use of isotopes, specifically the heavy isotope of oxygen, O^{18}, in water and carbon dioxide enabled Vinogradov and Teis [22] and, at the same time, the American scientists Ruben, Kamen, Randall, and Hude [23] to demonstrate that the isotopic composition of oxygen released during photosynthesis is the same as that of the oxygen of water and different from that of the oxygen of carbon dioxide. Therefore, this oxygen is the result of decomposition of water, and not of carbon dioxide. Thus, what had been for almost a century and a half an unproven hypothesis was conclusively demonstrated.

From this short account it is clear that the idea of the reduction of water during photosynthesis and the evolution of the oxygen formed had been advanced as early as the beginning of the nineteenth century. In the solution of the problem, scientists of many countries, including our own, participated. Russian workers played a prominent role, being responsible not only for the first correct guesses, but also for the conclusive demonstration of the hypothesis,using labeled atoms.

SUMMARY

One of the most important achievements in recent years in the study of oxidation-reduction processes of photosynthesis has been the final proof that the oxygen evolved as a result of this process comes from water. This discovery refutes the conception which was dominant beginning from the 19th century that the source of the oxygen is carbon dioxide of the air. An acquaintance with the work of a number of investigators of the 19th and beginning of the 20th centuries reveals that the conclusion of the modern scientists is not altogether novel and was previously held by many earlier scientists. Special investigations performed in the last century by Boussingault,Khodnev, Schmidt, Berthelot,and Bakh, and also by scientists at the beginning of the present century indicated that water used by the plants in organic syntheses was to a large extent, if not exclusively, the source of the oxygen evolved during photosynthesis. This opinion was supported by such famous scientists as Liebig, Erlenmeyer and Timiryazev. However,an irreproachable proof of this hypothesis could not be given at that time due to imperfection of the experimental technique. Only as a result of application of new methods and in particular, of the isotopic indicator technique has it become possible to prove the correctness of the conjectures of these scientists concerning the origin of oxygen from water. It should be noted that a large role in the elucidation of this problem was played by Russian scientists in the past as well as at the present time.

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BRIEF COMMUNICATIONS

ON CONDITIONS DETERMINING FLORAL BUD FORMATION IN POTATO

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The elucidation of the ontogenesis of potato in connection with vegetative reproduction is quite complex. To be specific, there are substantial differences in the course of individual development of plants from ordinary tubers and from seedlings. Under field conditions, plants from tubers begin to form floral buds about a month after tubers are set out. The period from sprouting to bud formation (appearance of floral primordia visible to the unaided eye) varies little from year to year. In variety Lorkh, for example, from 1951 through 1959 the length of time from setting-out to bud formation varied from 31.6 to 49.5 days, and the length of time from sprouting to bud formation, from 16.4 to 23.5 days. Bud formation occurs not only under normal field conditions but also in weak light [1], on a short day [2,3,5], and even in complete darkness [4, 5]. The dependence of bud formation in common varieties of potato on external conditions has been studied in greater detail by Jones and Borthwick [5]. They found that when the weight of tubers set out is kept down to 5 g, the temperature is increased, or the day length decreased to 9 hours, the number of nodes to the first inflorescence increases by about one node. This difference cannot be considered substantial, especially since with almost every treatment floral primordia formed even in total darkness.

On the other hand, seedlings often do not form buds in the year of sowing, and some of them do not flower for three [3] or more years. It therefore seemed that conditions promoting bud formation could be more easily determined in seedlings than in plants originating from tubers. To this end, the effect of temperature, rate of seed germination, and day length was studied.

Although experiments showed that seedlings which had been growing at a higher temperature, and also those which germinated earlier, began to form buds sooner, with every treatment individual seedlings were found which formed no buds at all. These conditions cannot therefore be said to be decisive in floral bud initiation.

Most interesting data were obtained with seedlings grown on a short 10-hour day. In 1953 an experiment was begun with a Majestic seedling (designated "seedling Z"). It was grown on a short day from the time of germination. Two shoots grew out from the axils of the cotyledons which, after rooting, were separated. One was left on a short day and the other was thereafter grown on a natural long day. In 1955 similar experiments were begun with three seedlings of a hybrid between Smyslov and Regina (designated "I", "O", "P"). They were also grown on a short day from germination, but it proved to be impossible to transplant the axillary seedlings for cultivation on a long day in the first year. The "long day" treatment therefore was effected by cultivation on a natural day of plants obtained from tubers in the second year. In the following years, seedlings obtained from tubers formed the previous year were grown at the given day lengths.

None of the four seedlings formed buds on a short day during the first year. Since the plants grown on a short day were weak, the tubers formed were small, but with each succeeding year the tubers became larger. As the table shows, bud formation by seedlings of vegetative origin on a short day occurred only when the parent tubers had attained a certain size. The smallest tuber weight at which bud formation first occurred was found in seedling "I". This is evidently related to the fact that it is the first to mature, judging by the bud formation of control plants in the previous two years. In seedling "P", which is the slowest to mature, even in 1959, when the tubers set out weighed 28 g, there were no buds formed. When plants were grown on a long day from tubers (average weight 16 g) which had been formed on a short day, however, bud formation began 60.7 days after setting out in 1959. Control plants (the whole year on a long day) began to form buds 44.3 days after setting out.

These data, indicating the necessity of a sufficient amount of nutrient materials for bud initiation, are in agreement with the observations of several years on the significance of tuber size. Because of the nature of this study, I was obliged to grow plants from small tubers for several years. The plants were strongly retarded with respect to floral bud initiation or formed no buds at all. In 1950, for example, Epron plants from tubers weighing 1.6 g formed buds 41.2 days after tubers were set out, while plants from tubers weighing 90 g formed buds in 23.5 days. When tubers were over 10 g in weight, the differences in time at which floral buds were formed were sharply reduced. In the experiments of Jones and Borthwick [5], potato plants of common varieties grown in complete darkness from tubers weighing 5 g formed no

TABLE. Floral Bud Formation by Potato Seedlings on a Short and a Long Day

Seedling designation	Year of seed planting	Bud formation on a long day in 1958 and 1959 (average No. of days from setting out of tubers)	First bud formation on a short day			Bud formation on a long day in the same year	
			year	tuber weight, g	No. of days from setting out of tubers	No. of days from setting out of tubers	tuber weight, g
Z	1953	29.6	1956	40	38	27	40
I	1955	28.5	1957	7	72	39	40
O	1955	33.2	1957	45	47	50	40
P	1955	48.3	–	–	None	–	–

buds, although plants from tubers weighing 15 or more grams laid down buds.

It must be noted that in other experiments in which plants were grown on a short day from tubers which had been formed on a long day, bud formation occurred if tuber weight was sufficiently high, as in the case of the experiments of Jones and Borthwick. In every case, however, the floral primordia failed to develop further on a short day. This was also observed by Surzhina [2] and Uspenskii [3]. Here it is evidently a matter not so much of the quality of the nutrient reserves but of their quantity. When tubers obtained from seedlings which had been grown on a short day are then set out and left on a long day, the resulting seedlings form buds and flower; this was observed in 1954 with plants from tubers weighing as little as 0.9 g. In this case the nutrient reserves were negligible, and bud initiation proceeds at the expense of assimilates produced by the growing plant.

These observations indicate that one of the decisive factors in floral bud initiation in potato is a sufficient supply of nutrient reserves or the possibility of synthesizing nutrients. (In the latter case bud initiation is retarded.) The failure of seedlings to flower in the first year of life is evidently a result of hereditary factors.

Surzhina [2] notes that when potato flowered on a 10-hour day the time at which flowering began was not influenced by the short day in the majority of cases. In our experiments, in which plants of common varieties were grown on a short day from tubers which had been formed on a long day, there was either no delay in bud formation, or if there was it was not greater than four days. Therefore, a short day does not substantially affect the time of bud formation and of flowering or the number of nodes to the first inflorescence.

However, during the first years the delay in bud formation in seedlings grown on a short day may be as much as 18 days. In the following years this difference subsides and even disappears completely. This can be explained by the fact that in the over-all cycle of development of the potato there is a juvenile stage (lasting about four or five years from germination).

It is well known that potato is a long-day plant. Razumov [6] states that with transfer to a short day there is an immediate cessation of flowering. We have observed that when a plant which had formed floral primordia on a short day was transferred to a long day the primordia did not develop further; only with further growth of the sympodial shoot on a long day were new inflorescences formed, which then flowered.

These facts concerning floral bud initiation by potato on a short day indicate that a long day is not necessary for completion of the light stage (in the sense of T. D. Lysenko), since in the presence of sufficient nutrient reserves it takes place under conditions as dissimilar as possible from those required for growth. This is in agreement with the data of Oleinikova [7] on the very early initiation of floral primordia in the growing point of plants being grown from tubers. A long day is therefore necessary only for growth of the inflorescence itself. Grebinskii [8] reports somewhat similar examples of the differing effects of day length on floral bud initiation and growth of inflorescences.

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BIOGEOCHEMICAL PROPERTIES OF SEVERAL SPECIES OF EREMURUS

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There are 23 species of the genus Eremurus (lily family) on the territory of the USSR, and 22 of these grow in Central Asia. These are ephemeral plants with a very short vegetation period: They flower in May and June at the time of abundant, but not continuous, rainfall, after which the dry period begins. In the spring the plants form a rosette of leaves, a stem, and a root tuber, in which a large quantity of nutrient substances are stored.

Species of this genus are differentiated by melliflu-ence and are highly decorative. However, interest in the genus Eremurus is also a result of the presence of adhesive substances in the root tubers that are of practical value.

In searches for new emulsifiers for pharmaceutical preparations, Ponamareva [1] separated from the Eremurus roots adhesive substances which are similar in external characteristics to gum arabic. Preliminary experiments carried out by B. N. Stepanenko and O. N. Ponamareva [6, 7] showed that the main part of these adhesive substances is a polysaccharide, which with iodine gives a red color similar to glycogen. This new polysaccharide was called eremuran. Its content constituted about 30% of the air-dry root tuber. O. N. Panamareva's investigations were made only with the species, Er. regelii Vved.

In connection with the multi-purpose characteristics of the species of genus Eremurus, the Flora Section of the Central Botanical Garden, USSR Academy of Sciences, set up a program to introduce and evaluate these species under cultured conditions.

In an experimental introduction of plants of various flora, in particular xerophytes from Central Asia, the necessity of studying the adaptation of the characteristics of these plants to the conditions of the soil environment was stressed. There are many notes on the chemical composition of various plants but in most cases the differences in chemical composition of the soils on which these plants grow are not known. On the other hand, numerous soil analyses were not accompanied by analyses of the chemical composition of the plant life growing on them [2, 3]. As a result, a series of observations of this type did not seem to be comparable to each other.

A significant decrease in the ash content of the roots of introduced plants in comparison to plants taken from natural conditions was noted in an experimental introduction of plants by the Flora Section of the Central Botanical Garden. Thus, Er. regelii introduced from Central Asia in 1957 had an ash content of 5.2%, but those plants brought earlier (1947) had an ash content of 2.8% under the effect of cultivation.

Such a change in the ash content is a result of the effect of climatic and soil conditions under cultivation.

In consideration of the necessity of combined analyses of the plant and the soil, spectrum analysis was made as a basis for comparison.

In order to characterize the mineral composition of the ash of natural and introduced plants, we made a spectrum analysis of the ash of the roots and leaves of various species of Eremurus. In addition to analysis of the ash of the leaves and roots, a comparative analysis of the soils from the habitat of the Eremurus of Kara-Bastau and soils of the cultivated plots at the Central Botanical Garden of the USSR Academy of Sciences was made.

Analyses were made on an ISP-22 spectrograph and used a constant-flow arc on spectrally pure carbon electrodes [4, 5]. The results of the analyses are presented in the Table. Twenty elements were observed on the spectrograms. Their concentration is conditionally characterized by the following signs: minus (-) shows that the lines of the indicated element are absent and its concentration in the sample lies beyond the limits of detection by spectrum analysis (without preliminary enriching of the sample). The sign "Tr" (trace) indicates cases that are positively detected although some of the last lines of the indicated element and its concentration appear approximately equal to the limit of observation.

Under the conditions of analytical work these limiting concentrations for observing the elements can be approximately estimated in percentages in the following manner: Mo—0.001%; Sn—0.001%; V—0.003%; Ti—0.003%; Al—0.001%; Fe—0.001%; Cr—0.001%; Ni—0.001%; Si—0.001%; Pb—0.003%; Sr — 0.0003%; Ga — 0.001%; Ag—0.0001%; P—0.1%.

Plus (+) corresponds to the presence of a large number of the last lines, which shows the concentration of the given element in quantities significantly exceeding the

Chemical Composition of the Ash of Roots and Leaves of Eremurus

No.	Plant name	Year of transfer from Central Asia	Cu	Mo	Sn	V	Ti	Ca	Na	Al	Fe	Cr	Ni	K	Si	Pb	Mg	Mn	Sr	P	Ag	Ga
								Ash of the roots														
440—41	Er. **regelii**	1947	++	Tr.	—	—	+	++	+	+	++	Tr.	—	++	+	+	+	+	+	+	—	—
2626	Er. **regelii**	1950	+	+	—	—	++	++	+	+	+	Tr.	Tr.	+	+	+	+	+	+	+	—	—
9107	Er. **regelii**	1957	+	Tr.	—	—	Tr.	++	++	+	+	Tr.	—	+++	+	+	+	+	+	+	—	—
438—39	Er. **olgae**	1947	++	+	+	—	+	++	++	+	++	+	Tr.	++	+	+	+	+	+	+	—	—
9105	Er. **fuscus**	1957	++	+	Tr.	—	+	++	++	+	+	Tr.	Tr.	+	+	Tr.	++	+	+	+	—	—
b/No.	Er. lactiflorus	1958	+	Tr.	—	—	+	++	++	+	+	Tr.	—	++	+	—	+	+	+	Tr.	—	—
								Ash of the leaves														
440—41	Er. **regelii**	1947	++	Tr.	—	—	+	++	++	Tr.	Tr.	—	—	+	Tr.	Tr.	+	+	+	Tr.	—	—
2626	Er. **regelii**	1950	++	Tr.	Tr.	—	Tr.	+	+++	Tr.	Tr.	Tr.	—	++	Tr.	Tr.	+	+	+	—	—	—
8648	Er. **regelii**	1956	++	Tr.	—	—	+	++	++	+	+	—	—	+	+	Tr.	++	+	+	Tr.	—	—
9107	Er. **regelii**	1957	++	Tr.	—	—	Tr.	++	+++	+	+	—	—	++	Tr.	Tr.	+	+	+	Tr.	—	—
438—39	Er. **olgae**	1947	++	—	—	—	+	++	++	+	+	—	—	++	+	Tr.	++	+	++	Tr.	—	—
9106	Er. **olgae**	1957	++	+	Tr.	Tr.	++	+	++	+	+	Tr.	Tr.	++	++	Tr.	+	+	Tr.	+	—	Tr.
4067	Er. robustus	1950	++	Tr.	Tr.	—	+	+	++	Tr.	+	Tr.	Tr.	++	Tr.	Tr.	+	+	Tr.	Tr.	—	—
8649	Er. robustus	1956	++	Tr.	Tr.	—	+	++	++	+	+	—	Tr.	++	+	Tr.	++	+	+	Tr.	—	—
6519—20	Er. altaicus	1956	++	Tr.	Tr.	—	+	++	++	+	+	—	Tr.	++	+	Tr.	++	+	+	Tr.	—	—
								Soil														
I	Soil from Central Botanical Garden		+	—	Tr.	+	+	++	++	++	++	+	+	+	+++	Tr.	+	+	+	Tr.	—	—
II	Soil taken from Central Asia (Kara-Bastau)		+	Tr.	+	++	+++	+	+	++	++	+	+	+	+++	+	+	+	+	Tr.	—	Tr.

limits of observation. Double plus signs (++) and triple plus signs (+++) indicate cases of a conspicuous richness of lines and their intensity. Boron lines are observed on all prints, but, because they are also present in the spectrum of pure carbon, appraisal of a difference in its concentration between separate views was not made.

Results of the analysis are of an orienting character, but make it possible to come to several preliminary conclusions having interest for further investigations.

The soil of Kara-Bastau is the richest of the soils of the Central Botanical Garden of the USSR Academy of Sciences in concentration of molybdenum, vanadium, titanium and gallium; calcium, sodium and strontium predominate in the soil of the Central Botanical Garden.

The ash of the leaves is differentiated from the ash of the roots in the concentration of macroelements and, in particular, of trace elements. It is notable that the ash of the leaves is somewhat richer in copper than the ash of roots and, on the other hand, the roots are richer in molybdenum than the leaves. The roots are richer in titanium, aluminum, iron, chromium, nickel, lead, strontium and phosphorus, and the leaves are richer in magnesium, potassium and sodium.

It is characteristic that vanadium, existing in abundance in the soils of Kara-Bastau, is hardly assimilated by plants, and traces of it are observed only in the leaves of a single species, Er. olgae, brought in 1956. The same applies to gallium, traces of which were observed only in the leaves of this plant.

From the data of this analysis, it follows that in the process of the plant's activity, molybdenum is energetically assimilated from the soil and its concentration in several cases, for example in the roots of Er. fuscus, and in the roots and leaves of Er. olgae exceeds its concentration in the natural soil. The same can also be said of the concentration of lead in the ash of the roots and strontium in the roots and leaves. Plants growing on soils with an insignificant concentration of molybdenum and strontium energetically accumulate them in their organs.

A richness of the ash of trace elements is observed for Er. olgae in relation to Er. regelii, when comparing individual species. This applies to Mo, Sn, Cr and Ni in the roots and Al, Fe, Si, Mg and Sr in the leaves.

Cases of a difference in concentration of individual elements were checked by control plates of corresponding objects.

Of the immediate practical conclusions that can be made on the basis of the analysis, one must stress the poorness of the soils of the Central Botanical Garden of the USSR Academy of Sciences in molybdenum and, obviously, the great demand for them by the plants, which are clearly enriched with molybdenum in relation to the soil feeding them.

I wish to thank E. Pedak, assistant in the department of analytical chemistry, Tartusk State University, for help in carrying out the spectrum analysis.

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DROUGHT RESISTANCE OF THE SIBERIAN APPLE AS A WILDING

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The Siberian apple has various uses in northern horticulture. It is natural material in the selection of winter-resistant varieties, is used in protecting field plantings, and serves in many places as a fruit tree and as an irreplaceable wilding for apples.

One of the necessary characteristics of a wilding, along with winter-resistant roots, is drought resistance. This question has general importance in many regions of Siberia and northern Kazakhstan in connection with the normal deficiency of moisture in May and June, when fruits and vegetative organs are developing intensively.

A number of horticulturists consider the Siberian apple a slightly drought-resistant wilding. At the same time other investigators say the opposite. These differences are connected with the fact that the Siberian apple is a collective term and includes a number of varieties with different drought resistance.

One of the goals of our work was to compare the Siberian apple with other species of apple according to the degree of resistance to inadequate moisture.

The work was carried out under the direction of Professor M. A. Lisavenko at the Altai Fruit and Berry Experiment Station.

The effect of soil drought on plants was studied according to the Rikhter method [1]. Leaf transpiration was measured under field conditions by the Ivanov method [2]. The study of the root systems of apple seedlings was carried out according to the Kolesnikov method [3], and the leaf area with a Dragavtsev drawing board [4]. For the tests we used: the wild Siberian apple (M. pallasiana Juz.) from the Zabaikal region; cultivated varieties of Siberian apple (M. baccata Borkh.); rennets, which are also related to the cultivated heavy-yielding variety of Siberian apple; forest apple (M. silvestris Mill.) from Voronezhsk district; Chinese apple (M. prunifolia Borkh.); seedlings of the heavy-yielding varieties (M. domestica Borkh.); Slavyanka and Pepin saffron, possessing different demands for air and soil moisture.

A reason for the weak drought resistance of Siberian apple is the character of its habitat (along river valleys), but it is thought that it cannot be grown in regions with light precipitation. However, this is not justified by the practical fruit growing of Siberia as a whole and Zabaikal in particular where seedlings of wild Siberian apple are the basic wilding. In these same places (Zabaikal) Siberian apple is grown successfully on the dry Aginsk steppe, in river valleys and on the mountain slopes [5].

Siberian apple, as V. M. Krutovskii noted, passed through the drought of 1910-1911 better than other trees and shrubs. The dry years of 1954 and 1956 also support the idea of its high resistance to air and soil dryness in the Altai region. In 1957 only 2 mm of precipitation fell in the Kulundinsk steppe from May 20 to June 27, but nevertheless the Siberian apple survived no less well than other forest species (hybrid poplar, yellow acacia, and silver oleaster).

Often the weak drought resistance of the Siberian apple is connected with the surface distribution of the roots, a result of its habitat in places with a high ground water table. The surface distribution of the roots of this apple in natural habitats is caused by low-vigor soils and mainly by their slow warming. Plants on cold soils distribute their roots in the surface levels where the warmer conditions are favorable [6]. In regions with inadequate moisture, a majority of the tree and grass plants also distribute their roots in the upper layers of the soil. This aids the interception of even a small quantity of the rainfall [8,9].

The distribution of the Siberian apple roots in the upper soil horizons according to Tanfil'ev [9] and Krutovskii [7] favors the simultaneous ending of growth in the autumn. Interruptions are not observed in the activity of the underground and above-ground organs early in the spring, and these can be observed in the case of deep penetration of roots into cold soils.

On relatively well-warmed and nonaerated soils with a rich chernozem layer, the Siberian apple roots are located in the lower and upper levels. Digging up of the roots of 120 rooted trees of 50-year-old garden Siberian apple and 2000 trees of heavy-fruited varieties grafted on this wilding was carried out in 1953 and 1954 in the Barnaul farm of the Altai Fruit and Berry Experiment Station. Of the 120 rooted trees of garden Siberian apple, 116 had roots in both a horizontal and vertical direction, penetrating into the soil to a depth of 2.5 meters. The main mass of the roots, as the digging up of the roots of eight-ten 16-year-old trees in 1956-1958 showed, are

located in the 10-20 cm level and significantly less of them in the 40-60 cm level.

Relatively strong development of the absorbing roots is also considered a sign of the weak drought resistance of the Siberian apple. Shitt [9] proposed that the wildings with large quantities of absorbing roots ensure the better growth of the tree in the vegetation period. According to his idea, the advantage of the Siberian apple over the forest variety would be maintained to a certain degree with drying of the upper layer of the soil. After this the tiny roots of the Siberian apple begin to dry more rapidly than the skeleton roots with a large cover of soil.

The skeleton roots of Siberian apple, as the investigations of the Altai Fruit and Berry Experiment Station show, cover a large volume of soil. At five to six years the trees grafted on seedlings of purple rennet or Siberian apple completely fill the garden rows to a width of 6 meters.

The drought resistance of Siberian apple can be judged also by the vigor of the root development in relation to the aerial organs [1]. This also is one of the indicators of a high resistance of plants to inadequate moisture. At the end of vegetation in 1954, one-year-old seedlings of wild Siberian apple had 2.59 g of roots per gram of absolutely dry weight of aerial organs, cultured Siberian apple 2.23 g, and forest apple 1.74 g. The vigorous development of the roots of Siberian apple seedlings assured their successful growth during the May-June drought in 1953 and 1955 when the seedlings of forest apple stopped growing.

The effect of drought on the roots of apple seedlings studied by the Rikhter method [1] showed that the wilting of leaves of all species takes place at 9% moisture in clay soil. Siberian apple seedlings were not differentiated from the seedlings of purple rennet, forest, Slavyanka and Chinese Sanin apple according to the degree of drying and falling of the leaves. Siberian leaves yield only to Slavyanka seedlings according to the quantity of healthy leaves retained.

After a period of ten days of soil drought, 14% of the seedlings of cultivated Siberian apple died because of death of the roots, Slavyanka seedlings 10%, forest apple 10%, and Saffron Pepin 40%. With the onset of optimal soil moisture the remaining healthy Siberian apple seedlings rapidly formed new leaves and absorbing roots. Thus, for example, the normal moisture of the soil in the containers was reached on August 28 and Siberian apple seedlings developed young shoots on September 10; this did not take place until September 24 for seedlings of the forest apple and Slavyanka.

We know that plants with a large water-retention capacity of their colloids economically consume moisture and are more resistant to drought. After eight hours, dried wild Siberian apple lost 33% of the original quantity of moisture, cultivated Siberian apple 39.1%, forest apple 30%, Chinese Sanin 39.3%, eastern apple 39.1%, and purple rennet 44.2%. Thus, seedlings of Siberian apple somewhat yield to seedlings of forest apple based on water-retention capacity but are not differentiated from Chinese and rennet seedlings. Basic differences were not noticed in the evaporative ability of leaves of apple wildings. After five minutes, seedlings of the wild Siberian apple evaporated 0.42 g of water per 100 cm^2 of leaves, cultivated Siberian apple seedlings 0.37 g, forest apple 0.42 g, Sivers apple 0.35 g, and Chinese Sanin 0.45 g.

These physiological indicators are presented for plants with roots not damaged by low temperatures. After wintering, roots of all wildings with the exception of Siberian apple are usually damaged to some degree. In the dry regions of the Kulundinsk steppe and in Northern Kazakhstan, the high resistance of roots of Siberian apple to low temperatures also aids the increase in its drought resistance. The middle Russian, Michurinsk and local varieties grafted to Siberian apple use the spring reserves of soil moisture productively and give a high yield and rich growth even in years with inadequate moisture. This is explained by the fact that the healthy roots of the wilding absorb moisture intensively immediately after snow removal and contribute to the high assimilation energy of the wilding.

All that has been presented does not give a basis for considering Siberian apple a slightly resistant wilding. Wild varieties of Siberian apple used in regions where the climatic conditions do not correspond to the natural rhythm of the species develop poorly and as a result of this survive drought poorly. Siberian apple and especially its cultivated local varieties in the steppe regions of Siberia and Northern Kazakhstan are sufficiently resistant to drought, and this supports the feasibility of their growth in these places.

One must also stress the fact that other fruit species, with the exception of the steppe cherry, cannot grow successfully in droughty regions of the Kuludinsk steppe without additional moistening of the soil during the summer period. This is done either by selection of an area where ground water is nearby and much snow is accumulated, or by irrigation of the fruit and berry plants in the most favorable phenophases of development.

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THE EFFECT OF INJURY ON THE GREENING OF ALBINO SUNFLOWER PLANTS

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In one of the works [1] that we published earlier, we noted that cases of greening of albino sunflower plants were observed soon after removal of their roots. The indicated facts prompted us to carry out more basic investigations in this direction. Albino sunflower plants with green cotyledon leaves for which the following real leaves were lacking chlorophyll and complete albinos for which the cotyledon leaves did not contain chlorophyll were used to study this problem. The scheme of the experiments was as follows: The roots were removed from one portion of the plants, after which the plants were placed in a container with water; the rest of the plants were severed above the cotyledon leaves, after which these plants were also placed in a container with water. The part of the plants severed above the cotyledon leaves as a rule had two or three pairs of real albino leaves. In the third series of experiments, the tips were cut off albino plants that were grown in flower vases, and that had formed two to three pairs of albino leaves.

First we will look at the results of the removal of the roots of albino plants whose cotyledon leaves are green (Table 1). In 11 cases out of 12, we observed greening of the plants six to 20 days after removing the roots. For plants that had a developing second pair in addition to a pair of formed leaves at the time of removal of the roots, the former greened more intensively as a rule, a fact suggesting a more rapid movement of this process in the young forming parts of the plant. This process takes place more rapidly (six to seven days) for the younger 12-day-old plants at an increased temperature of 25-28° (Numbers 9, 10, 11, 12). These same plants also die more quickly. Greening at a temperature of 15-18° begins on the 13th-20th day after removal of the roots for plants at an age of 19-28 days. The exception was plant No. 8 (see Table 1) for which the greening began on the seventh day.

We will shift to a look at this process for complex albino plants. The data for these plants is presented in Table 2. In eight cases out of 11, greening was observed for the complete albinos after removal of the roots. For two plants, there was no greening because these plants died on the third day after removal of the roots. In one case greening was absent for a 23-day-old plant. Greening for most plants began on the sixth to seventh day. Only for one plant (Table 2, No. 11) did greening begin on the 11th day. The delay noted in the onset of greening was connected with decreased temperature (15-18°) where the plant was left after root removal. If the data in Tables 1 and 2 are compared, it is quite obvious that this process takes place more rapidly when the operation is carried out in June or July when the air temperature is sharply increased (25-28°).

Data presented in Tables 1 and 2 conclusively show that relatively rapid greening of the plants begins as a result of root removal. This is connected with the removal of the roots as a result of which, it must be assumed, the normal activity of the plant organism is strengthened, and in the end this leads to a serious shift of its activity and activates processes promoting the formation of the green pigments. Greening was not observed for control plants with roots, as Table 1 and 2 indicate.

No less interesting in this context are the experiments on removal of the uppermost point of growth of the albino plants. The upper points of growth were removed for 52-to-54-day-old plants, which at this time in the spring had formed five pairs of albino leaves under greenhouse conditions at a temperature of 10-20°. On the seventh and eighth day after the growing tips had been removed, the two uppermost pairs of leaves also began to green very intensively. It is necessary to note that seven to ten days after the removal of the tips albino shoots began to develop from the notches of the upper green leaves and only after ten to 15 days was weak greening observed. While observing this, we decided to remove the upper point of growth of albino plants that were comparatively well greened. Over 20 such plants of different ages were taken and in all cases shoots devoid of chlorophyll developed from the bud notches of the upper leaves after seven to ten days, and only after eight to ten days did they gradually become green. This suggests that the metabolism of the albino plants, with a complex of favorable conditions leading to their greening does not induce dormant shoots in the leaf notches. Only after their activation and some period of development are they included in the general process of metabolism

TABLE 1. Development of Albino Sunflower Plants After Removal of the Roots

Plant number	Age (days)	Degree of development	Date of removing roots	Number of days after removal of roots that greening began	Parts of plant beginning to green	Number of days after removal of roots that plant died	Notes
1	28	First pair of real leaves formed. Beginning of formation of second pair	3/12	13	First pair of leaves along the central vein	22	Cotyledons died first
2	23	First pair of real leaves formed	3/5	20	Same	24	Same
3	23	Same	3/5	20	First pair of leaves from the petiole	24	"
4	23	"	3/5	–	–	25	No greening
5	23	First pair of real leaves formed Beginning of formation of second pair	3/5	20	First pair; second pair more intensively	30	Cotyledons died first
6	23	Same	3/5	20	Weak greening of first pair. Second pair greened more intensively	30	Same
7	19	First pair of real leaves formed	3/12	18	One-third of leaf from the petiole	27	Cotyledons died first
8	19	First pair of leaves formed. Beginning of formation of second pair	3/12	7	First pair	–	Accessory roots formed on the 25th day
9	12	First pair of leaves formed. Beginning of formation of second pair	7/9	7	Second pair of leaves	9	–
10	12	Same	7/9	7	Same	9	–
11	12	"	7/9	6	"	8	–
12	12	"	7/9	7	"	9	–

TABLE 2. Development of Complete Albino Sunflower Plants After Removal of the Roots

Plant No.	Age (days)	Degree of development	Date of removal of roots	Number of days after removing roots that greening began	Parts of plants beginning to green	Number of days after removal of roots that plant died
1	12	First pair of real leaves formed Rudiments of second pair	7/9	7	Second pair of leaves	9
2	12	Same	7/9	7	Same	9
3	12	"	7/9	6	"	8
4	12	"	7/9	7	Second pair and rudimentary third pair	13
5	12	"	7/9	7	–	3
6	12	"	7/9	5	Second rudimentary pair	7
7	10	"	7/9	–	–	3
8	5	Cotyledon leaves formed	6/17	6	Cotyledons	10
9	5	Same	6/17	6	Same	10
10	23	Cotyledon leaves formed Rudiments of first pair of leaves	8/12	–	–	20
11	11	Cotyledon leaves formed	8/12	13	Cotyledons and first rudimentary pair of leaves	20

and do their shoots begin to green. Much factual material which we are presenting in this paper gives a basis for asserting that as a result of injuring albino plants (removing roots or tips of the point of growth) a relatively rapid greening of the albino leaves, regardless of the part removed (roots, or stem tips) is observed, and this greening occurs most intensively for the young leaves. We must assume that this is connected with the activation of the hormone activity of the plants, which plays a necessary role in the formation of the green pigment.

One can suggest a similar character for the metabolic processes, which scarcely at all induce the formation of albino shoots for well-greened albinos in the dormant parts of the plants (dormant shoots). Only some time after the dormant shoots begin to grow are they included in the normal chain of metabolic processes taking place in plant organisms, which in the end lead to greening of the albino shoots from the developing buds.

The data that are presented suggest the enormous effect of injury on the greening of albino plants, in all probability connected with the strengthening of hormone activity in these plants.

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*See English translation.

A FIELD TRANSPIROMETER

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By far the most widely used method for measuring transpiration is the determination of water loss from detached plant leaves. The details of this method have been very carefully worked out in recent times [1]. However, this method is of little use for field measurements, not to mention the fact that we cannot measure the transpiration of the same leaf for protracted periods, and therefore it is not possible to study the dynamics of leaf transpiration.

There are methods which permit the study of transpiration in an air current. For example, in one of these methods the luminescence of moist air in a beam of radioactive and ultraviolet wavelengths is used [2]. A hair hygrometer described in a work of Nikolaev [3] and Votchal [4] is also used for measurement of transpiration.

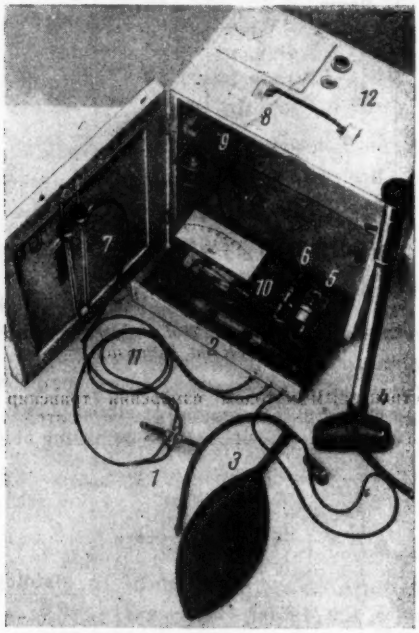

Figure 1. Disassembled view of the transpirometer. 1) Leaf chamber 2) Indicating hair hygrometer 3) Rubber air bladder 4) Air pump 5) Reducer 6) Air escape needle valve 7) Flow meter 8) Box 9) Air moistening tube 10) Thermometer 11) Rubber tubing 12) Level.

In 1955 we designed and constructed a transpirometer weighing 4.8 kg for measuring transpiration in a current of air.

PRINCIPLES OF THE METHOD

Air with a known relative humidity and moving at a certain constant speed is passed directly through the chamber containing the leaf. After passing through the leaf chamber the air passes through a sensitive, indicating hair hygrometer. If the increase in relative humidity of air which has passed through the leaf chamber is known we can calculate the amount of water transpired by a unit area of leaf surface.

Since the hair hygrometer has little inertia, the apparatus can measure transpiration directly. It can also measure rapidly and reliably the transpiration of plants being compared. Because of simplicity in construction and inexpensiveness several of the apparatuses can be used simultaneously. The task of the operator is primarily one of observing the pointer of the hygrometer indicator.

CONSTRUCTION

The twelve parts of the transpirometer are shown in Figures one and two. The leaf chambers consist of two halves with areas of 0.5 or 1 cm^2.

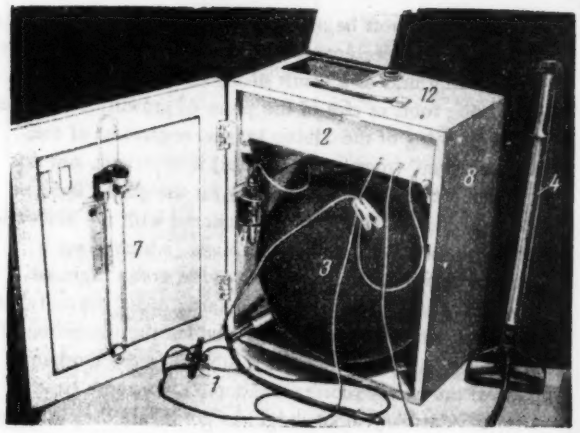

Figure 2. View of assembled transpirometer (legend as in Figure one).

TABLE 1. Weight of Water Vapor in Saturated Air at Various Temperatures (grams/m^3)

T°	Weight	T°	Weight	T°	Weight	T°	Weight
0	4.84	11	10.01	23	20.57	35	39.59
1	5.19	12	10.66	24	21.78	36	41.70
2	5.55	13	11.34	25	23.04	37	43.90
3	5.94	14	12.07	26	24.37	38	46.20
4	6.36	15	12.83	27	25.77	39	48.60
5	6.79	16	13.63	28	27.23	40	51.11
6	7.26	17	14.48	29	28.76		
7	7.75	18	15.37	30	30.37		
8	8.27	19	16.31	31	32.05		
9	8.82	20	17.30	32	33.81		
10	9.40	21	18.33	33	35.66		
		22	19.43	34	37.58		

The hair hygrometer is a fundamental part of the apparatus. It is designed to measure the relative humidity of an air current. For this purpose hair washed free of oil with ethyl ether for four hours is fixed in a glass tube. The indicator of the hygrometer is made of aluminum foil suspended by cementing it with BF-2 glue to a strand of polyamide plastic 0.1 mm in diameter. The hygrometer scale is proportional. A calibration curve (Fig. 4) is used for translating the scale reading into percent relative humidity. A special threaded base with a spring permits the glass tube containing the hair to be traversed.

The rubber bladder from a soccer ball is used. Air is pumped through a nipple into the bladder with a bicycle pump until it has a volume of 4-5 liter. Other rubber bladders having larger volumes can be used.

A reducer automatically regulates the air speed to 3 liters/hour. In structure it resembles the ordinary membrane gas-reducer except for its smaller size. The ventilating needle valve also serves as an air speed regulator. The final regulation of air speed is accomplished with a needle valve and a kerosene flow meter. A variable resistance serves as an occasional controller for regulating the reducer and permits the air speed to be varied from 0-4 liters/hour. A single regulator can serve three or four transpirometers.

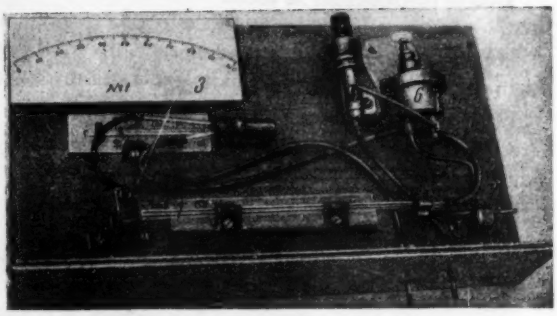

Figure 3. Hygrometer. 1) Glass tube containing the hair 2) Support for the indicator needle 3) Indicator needle 4) Thermometer 5) Exit needle valve 6) Reducer valve 7) Device for varying tension on the hair.

All the parts of the apparatus have been assembled in a wooden box painted white. The pointer of the hygrometer can be observed through a glass window in the top of the box. The window is covered with a special lid.

The vessel for moistening the air is depicted in Figure 5. It serves to moisten the air to 100% relative humidity in order to adjust the pointer of the hygrometer to 100%. A thermometer is mounted in the vessel through which passes the air being analyzed by the hygrometer (see Fig. 3). The temperature is read at the same time as the relative humidity of the air. All parts of the apparatus are joined by rubber tubing. It is necessary to use small diameter rubber tubing (e.g. nipple tubing). With a smaller system volume the inertia of the apparatus will be less, and less gas volume will be needed for measurements and flushing the system.

The level helps in placing the apparatus in an exactly horizontal position. In addition, the glass tees which are used for convenience in joining the rubber tubing during changes are a basic detail of the apparatus.

METHODS OF MEASUREMENT

The apparatus is adjusted before measurements are made. We recommend that the viewing window in the

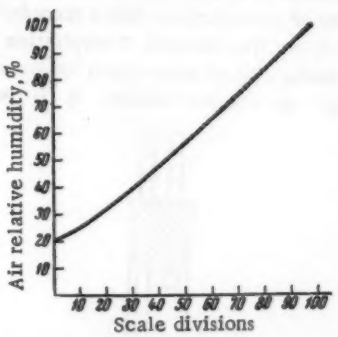

Figure 4. Hygrometer calibration curve. 1) Air relative humidity, %; 2) Scale divisions.

TABLE 2. Transpiration of Potato Measured by the Transpirometer and Weight Methods.

No.	Locality	Transpirometer g/dm²/hr	Weight method, g/dm²/hr
1	Greenhouse, pots	0.46	0.56
2	" "	0.51	0.67
3	Field plots	1.92	2.16
4	" "	2.9	3.26
5	" "	3.5	3.2
6	" "	6.0	4.05
7	" "	6.3	4.23

box be covered to shield it from the direct rays of the sun. Air is pumped into the rubber bladder. The bladder occupies a certain volume limited by the size of the box, so that it holds about five liters of air. Then the air is moistened in the vessel containing water. The moistened air is passed through the hygrometer. The box is leveled and the hair hygrometer is adjusted by a special device so that the hygrometer pointer reads 100. Afterward when the apparatus is moved it is placed only in a horizontal position.

After the apparatus is adjusted the system is flushed with fresh natural air for 15 minutes. The regulator automatically regulates the speed to 3 liters/hour during all the changes.

After the relative humidity of the original air is determined the chamber is quickly fitted onto the leaf of the experimental plant. Observations can be made 10-15 minutes after the chamber is fastened to the leaf. The data can be recorded every minute or for a five-minute period depending on the problem at hand. The apparatus has little inertia so that changes in needle position can be observed even over half-minute periods. When observations are being made of transpiration rates during short periods of time, a record should be taken every minute. If it is necessary to make prolonged observations of transpiration, then a recorded observation is made every five minutes. Transpiration measurements can be made without interruption for an hour without pumping up the air bladder. It is essential that a constant air speed be maintained. After each inflation of the bladder the relative humidity of the air should be determined.

Figure 5. Air moistening vessel.

TRANSPIRATION CALCULATIONS

Transpiration can be calculated by the equation:

$$T = \frac{C \cdot K \cdot P \cdot (B_T - B_O)}{100\ 000},$$

where: T = transpiration of water, grams/dm²/hr;
C = air speed, liters/hr;
K = transference coefficient = $\frac{100^2}{\text{area of leaf chamber, cm}^2}$;
P = weight of water vapor in air saturated at the experimental temperature, g/m³ (see Table 1);
B_O = relative humidity of original air, %;
B_T = relative humidity of air emerging from the leaf chamber, %.

HYGROMETER CALIBRATION

First of all, the hygrometer indicator must be set on the hundredth division. To do this it is necessary to put moistened air through the apparatus for 15-20 minutes and by altering the tension on the hair to set the needle indicator on 100. This should correspond to an indicator setting of 100% relative humidity. The rest of the calibration of the hair hygrometer can be done with an Assman psychrometer. Air with a certain relative humidity is passed through the chamber and passed by the hair hygrometer for 15 min. After observation of the transpirometer indicator position the air is analyzed with an Assman psychrometer. The air from the rubber bladder is led through the rubber tubing and two stoppers containing holes to the thermometer of the psychrometer. The relative humidity of the air is then analyzed by the Assman psychrometer as usual.

Usually it will be sufficient to obtain 3-4 values at different humidities in order to plot a calibration curve, since the curve closely approximates a straight line (see Fig. 4).

COMPARISON OF TRANSPIRATION DETERMINED BY THE TRANSPIROMETER AND WEIGHT METHODS

Potato plants were used for comparing the measurement of transpiration by the two methods.

Transpiration was measured under both field and greenhouse conditions. In the weight method transpiration was determined as the average of weighings of four leaf discs. Discs were cut from the same leaf to which the transpirometer chamber was attached. The transpirometer indicator was read each minute for 10-12 minutes. Trans-

piration was determined by both methods at the same time. Average results are shown in Table 2.

The data in Table 2 show that at low transpiration rates the weight method gives slightly higher values than the transpirometer. This is related to the reaction of the plant to injury. At average values of transpiration the transpirometer readings nearly correspond with those obtained by the weight method. At high rates of transpiration the values obtained by the weight method fall below those obtained by the transpirometer. This is explained by the fact that a leaf disc cannot transpire greatly because water influx is prevented. We can conclude that the transpirometer method is more exact than the weight method since the plant remains intact when the transpirometer is used.

SUMMARY

The main part of the transpirometer consists of a sensitive indicating hair hygrometer suitable for measuring the relative humidity of an air current. A reducer automatically regulates the pressure in a rubber chamber. As a result of the factor of constant velocity, the transpiration can be calculated by a formula taking into account velocity and temperature of the air, area of the leaf chamber, and the difference in the relative humidity of the air input and output. In contrast to the weight method, the transpiration of undetached leaves is measured, and therefore our method is the more accurate.

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BOOK REVIEW

S. S. Andreenko and F. M. Kuperman,
The Physiology of Corn.
Outlines in physiology of development, growth, photosynthesis, mineral nutrition and water regime.
Izd. Mosk. In-ta, 1959. 18, 27 signatures. Edition of 5000 copies.

Reviewed by V. P. Dadykin

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The cultivation of corn which is of great and constantly growing significance to the country's agriculture, altogether justifies the appearance on the bookshelves of a reference work on the physiology of this crop. Undoubtedly, interest in a many-sided study of the biology of corn will be growing further. Thus, a summary of the available data on the physiology of this plant becomes all the more necessary. In their preface, the authors note with regret that corn has relatively seldom been used as an object for physiological studies, and this circumstance explains the fragmentary nature one notes in the separate parts of this book.

Evidently because of this circumstance, as well as because of a quite understandable and excusable caution, the title of this book is provided with the subtitle: "outlines of physiology of development, growth, photosynthesis, mineral nutrition, and water regime." It seems as if it would not have been a mistake on the part of the suthors to leave on the title page only the subtitle, raising it to the rank of a title. The content of the book corresponds more to an "outline." However, this is not so much the fault of the authors, as the fault of the limited data existing in the literature for a systematic and exhaustive examination of all problems of corn physiology.

The book contains the following chapters: 1) botanical description of corn, 2) some problems of ecology and corn cultivation, 3) physiology of corn seeds during the period of their growth and storage, 4) water regime of corn, 5) mineral nutrition of corn, 6) photosynthesis in corn as the basis of its yield, and 7) morphological characteristics of the growth and development of corn. By this plan, as can be seen from the chapter headings, the most important problems of the physiology of the corn plant are covered.

A somewhat different impression is left, however, when the contents of the chapters are examined. One may feel that the book was written with a certain amount of haste, which is revealed in the incomplete survey of the literature sources, and occasionally in unnecessarily generalized formulations, incomplete elimination of redundancies in the manuscript, etc.

Let us consider the second chapter, which begins with a mention of the fact that corn has recently become widely cultivated in our country in various geographic regions with very different soil and climatic conditions. The interest of the reader in the contemporary area of cultivation for this crop and in the characteristics of its behavior in different regions is natural and legitimate. But the authors restrict themselves to the general statement: "Corn may grow and give high yield of green mass and ears in various climatic and soil regions" (page 4). Even a person who is little interested in the problems of corn cultivation may easily realize that it is one thing to grow this crop in the Kuban region and quite a different thing to grow it near Leningrad, where corn is also cultivated in recent years. The properties and peculiarities of ecological conditions for corn—considering the whole great area of its distribution and indicating the specific properties of agrotechnical conditions in each region (especially in those regions where it has been introduced only recently)—should, it seems to us, be the basis of the content of this chapter. However, the whole chapter—as a matter of fact, the whole book—tells about physiological processes affecting the cultivation of corn in general, and not as applied to definite geographic, soil, and climatic regions.

This significant omission of the authors may be illustrated by a number of examples. "These examples indicate that corn may be cultivated on the most different soils" (page 40). Insofar as the use of mineral fertilization for corn is concerned, it should be pointed out that the introduction of "superphosphate phosphorus flour, phosphate cinder, ammonium nitrate, kainite" is very effective (page 51). Where, on which soils, or in which regions, predominantly these or the other fertilizers are needed is not indicated.

The indications concerning the part played by soil temperature during the spring period are very imprecise (page 41). Recently, data have appeared concerning the

very unfavorable effect on the plantings of low soil temperature when combined with high humidity, and about relatively favorable survival of planted seed at lower soil temperature when there is no simultaneous excessive irrigation. The authors do not mention this.

Low soil temperature during planting retards the appearance of seedlings. This is a well-known fact—known long ago. But at present, there are data indicating that plants in which the appearance of seedlings was retarded as a result of low soil temperature during planting later not only catch up with but actually surpass in their development plants of later plantings. These facts, in part, we have observed while working with corn in Yakutiya. But the physiological basis for this phenomenon as yet is not discovered. Other technical conclusions from these facts are that one should not wait till soil is warmed up to 10° when cultivating corn in northern regions, since this is not justified from the agricultural point of view.

These problems were considered separately in the work of regional institutions (Komi ASSR, Tyumenskaya region, Irkutsk, Yakutiya and others), but the authors do not consider these. Because of this, it would have been very desirable to generalize the experience relating to the time of planting in this northern region of corn cultivation, and to submit this experience for the consideration of physiologists.

In connection with this circumstance, the mention of the authors that "the corn yield is created in the second half of vegetation" (page 58) seems to invite caution. Such a hypothesis may give an incorrect orientation for the agronomist. There are places in which the second chapter resembles an agronomy manual. However, the agronomy manual turns out to be too generalized for the scale of the whole country, and because of this, occasionally the value of the recommendation is doubtful. For example, it was pointed out that "the best period for the autumn ploughing for corn is considered to be July-September" (page 49). "The best times of planting are determined specifically in each region and depend on a combination of all climatic conditions of the given year, and the quality of soil and seed" (page 56).

The other chapters of this book are also not free from places which do not help much in understanding the essence of the physiological processes of corn cultivation. For example: "When light is not sufficient and with lowered temperatures, sometimes anomalies arise in development of male and female flowers" (page 65). How insufficient is illumination, which anomalies in flower development are concerned, and how to understand "sometimes" is not indicated in the book. Without explanation of these unclear statements, this information cannot be actively used by the investigator-physiologist or the practical agronomist.

The data concerning the possibility of using immature seeds for planting are significant and interesting. It is a pity that physiology of storage of seeds shelled and on the ear, and the role played by the temperature of storage are not discussed.

In the chapter about the water regime of corn, the authors begin by recognizing the decisive role of osmotic forces in the process of water absorption, and find it possible not to mention the theories developed mostly by the Kazan School of Physiologists (Alekseev, Gusev), according to which the decisive role in the process of water absorption is played by the hydration of colloids and thermodynamic processes.

The attempt of the authors to equate the combination of temperature and moisture optimal for the conditions of the corn belt of the USA with conditions in our country (page 112), makes one cautious. If analogies of that kind are given, then, at least, climatic indicators should have been given.

It is hardly possible to agree with the theory of the authors that the most important consequence of lowered temperatures in a physiological sense is the decrease in kinetic energy, increase in protoplasm viscosity, and decrease in solubility of some of the substances and in rate of their diffusion. As a result "the rate of respiration and translocation of substances in the plant may be decreased, while retardation of these processes in turn limits the amount of energy in roots and the amount of food necessary for root growth" (page 135).

There are experimental data in the literature concerning the activation of respiration processes at lowered temperature of the medium. Also, since the end of the last century it has been known that the roots grow more extensively at lower soil temperature than during high temperature (Bialoborry, Kosaroff, Tol'skii, Radchenko, Dadykin Korovin and others). Recently there are more and more factors which indicate more active absorption of the sunlight energy by plants growing at lower temperatures.

Very little is said in the book about the utilization of light energy by corn grown under different conditions.

Haste in the preparation of the book is also indicated by editorial correction which is not always careful. Thus, "Soil with shallow deposit of soil layer" occurs as a sentence (page 49). A unit of measurement is not given in some tables, which makes them little intelligible (Table 46). Sometimes essential items are missing in the exposition. Example: In Table 60, concerning the effect of watering on maize yield, the norm of watering is not indicated.

In the beginning of the last chapter the authors insist with complete justification that "it is necessary to study carefully in each specific region of corn cultivation, in which variety, under which specific conditions and at which stage of development, at which age of the plant, and in which stage of ontogenesis the yield is formed in a proper direction" (page 169). Unfortunately the authors do not always succeed in observing this rule in presenting the material; because of this, their work is in some ways unsuccessful.

Nevertheless, in spite of the deficiencies of the book, its publication is a welcome event. Anybody working with corn obtains thereby a summary of the basic results of investigations on the physiology of this crop, and a substantial (although not exhaustive) bibliography.

The publication of this book raises this question: Is it not time, in our country, to create a number of monographic summaries of the physiology of the most important cultivated crops and the most important forest-forming species? It seems that the appearance of such summaries would have facilitated the work of numerous agronomists, selectionists, biochemists, geneticists, foresters, and others.

CURRENT EVENTS

EIGHTIETH ANNIVERSARY OF THE BIRTH OF ACADEMICIAN N. A. MAKSIMOV

V. N. Zholkevich

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The 80th anniversary of the birth of the renowned scientist – plant physiologist Academician Nikolai Alexsandrovich Maksimov fell on March 21, 1960.

On that date a special session was convened of the Scientific Soviet of the K. A. Timiryazev Institute of Plant Physiology, USSR Academy of Sciences, whose director, until the last days of his life, was N. A. Maksimov.

The introductory remarks were made by Academician A. L. Kursanov. He emphasized the enormous influence exerted by N. A. Maksimov on the development of physiological investigation in our fatherland. Maksimov laid the foundations of drought-and frost-resistance studies, and essentially became the founder of ecological plant physiology. N. A. Maksimov also accomplished much in developing studies on growth stimulation, on illumination in plant cultivation, on physiology of irrigated cultivations, and on a number of other physiological problems.

I. I. Tumanov, an associate member of the Academy of Sciences, presented a report of studies by N. A. Maksimov on plant frost-resistance; P. A. Genkel', an associate member of the Academy of Pedagogical Sciences, RSFSR, reported on the value of studies by N. A. Maksimov on developments in water management and drought-resistance of plants. The speakers, after a thorough analysis of scientific and pedagogical activities of N. A. Maksimov characterized him not only as an outstanding scientist, but also as a remarkable human being.

N. A. Maksimov, a pupil of V. I. Palladin and D. I. Ivanovskii, began his studies by investigating plant respiration. However, in subsequent studies he became greatly interested in natural plant environments. Engrossed in his studies of ecological physiology, Maksimov conducted most interesting investigations which led to novel ways of resolving the whole problem of plant drought- and frost-resistance. His main studies in this field, which are still of value today, can be considered truly classic.

The role of N. A. Maksimov as an organizer of scientific pursuits in this country was extraordinarily great. He directed university studies in many USSR cities, and in his last years was chairman of the Department of Plant Physiology in the Timiryazev Agricultural Academy. He established a number of physiological laboratories. For 10 years the plant physiology laboratory of VIR [All-Union Inst. of Horticulture] was, under the leadership of N. A. Maksimov, the leading laboratory of the country.

N. A. Maksimov contributed enormous efforts to the consolidation and development of the K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences, first as its director pro tem. (beginning in 1943) and later (from 1945-1952) as director. He was the initiator in building the Station of Artificial Climate.

N. A. Maksimov is the author of the best widely used textbook on plant physiology, which had eight editions during his lifetime and was published as the 9th edition in 1958 under the editorship of A. L. Kursanov. Written in fine literary style (as, by the way, were all the other studies of Nikolai Aleksandrovich), the "Brief Course on Plant Physiology" was translated into many languages of the USSR, as well as into English, German, Japanese, and Spanish. The name of its author is known throughout the world. In 1946 the seventh edition of the textbook was granted a K. A. Timiryazev award.

N. A. Maksimov founded a vast school of plant physiologists, which trained many valuable specialists. He adhered to an unusual method of training, which proved itself. Usually Nikolai Aleksandrovich did not hamper the beginner by excessive control, but gave him considerable independence. Nikolai Aleksandrovich would say that in principle he disliked interfering in a person's work, that he disliked "leading a person by the hand." And yet at a difficult moment one could always appeal to him for advice, and Nikolai Aleksandrovich would always indicate the correct solution.

A human being of rare cordiality, exceptionally kind and responsive, Nikolai Aleksandrovich conquered by his simplicity and spontaneity in handling those surrounding him. In conversing with him, people felt remarkably free. One could argue freely with Nikolai Aleksandrovich; he never demanded full agreement. Tolerance in relation to the views of other investigators, evaluation of their experimental data and their conclusions without bias, constituted his characteristic trait.

People from different parts of the USSR frequently appealed for consultations with N. A. Maksimov, the most prominent scientist, the acknowledged head of Soviet physiologists. Despite the fact that he was a very busy man, Nikolai Aleksandrovich found time for all who appealed to him, endeavoring to help by kind advice and direction.

All who knew N. A. Maksimov always retained the very finest memories of him as a great scientist and a charming human being.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

*BRITISH-AMERICAN TRANSLITERATION SYSTEM.

Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.